Journal of Chemical and Pharmaceutical Sciences

Journal of Chemical and Pharmaceutical Sciences-JCHPS (2025) 18 (4): 84-94
Online ISSN: Online 2349-8552, Print ISSN: 0974-2115
Research Article | Pharmaceutical Sciences | MCI Approved | Peer-Reviewed | Referred | OA Journal |

# Phytochemical Characterization and Standardization of *Ipomoea parasitica* Aerial Parts: Successive Soxhlet Extraction, Total Phenolic and Flavonoid Quantification, and HPTLC Fingerprinting

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Received: 22 Aug 2025 / Accepted: 10 Sept 2025 / Published online: 01 Oct 2025
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## ABSTRACT

*Ipomoea parasitica* (Convolvulaceae) aerial-part phytochemistry diverges markedly from the resin-glycoside-dominated seed and latex chemistry of the same species. The present study establishes a multi-stage quality framework for the dried aerial-part powder, documents successive Soxhlet extraction along a hexane–ethyl acetate–ethanol–hydroalcoholic polarity gradient, and characterizes the resulting four fractions through qualitative phytochemical screening, Folin–Ciocalteu total phenolic content (TPC), aluminium chloride total flavonoid content (TFC), and HPTLC fingerprinting with densitometric quantification of three polyphenolic markers. Raw-material pharmacopeial parameters — foreign matter 0.6 ± 0.1% w/w, loss on drying 7.8 ± 0.3% w/w, total ash 9.2 ± 0.4% w/w, acid-insoluble ash 1.4 ± 0.2% w/w, water-soluble extractive value 18.7 ± 0.8% w/w — fell within pharmacopeial limits. Soxhlet yields followed the polarity gradient: hexane (HE) 2.4 ± 0.18%, ethyl acetate (EAE) 4.7 ± 0.31%, ethanol (EE) 7.9 ± 0.42%, hydroalcoholic (HAE) 6.3 ± 0.36% w/w. The ethanolic fraction carried the highest TPC (78.3 ± 3.0 mg GAE/g), TFC (56.9 ± 2.5 mg QE/g), and HPTLC marker load: gallic acid 1.18 ± 0.05 mg/g, rutin 2.34 ± 0.09 mg/g, quercetin 3.12 ± 0.11 mg/g. Inter-batch reproducibility of the lead ethanolic fraction across three independent harvest campaigns returned RSDs of 2.2–4.4%, within all acceptance windows. The aerial-part flavonoid-and-phenolic-acid-dominated profile places *I. parasitica* within the antidiabetic-active stream of the Ipomoea genus and supplies a standardized chemical input for downstream pharmacological evaluation.

**KEY WORDS:** *Ipomoea parasitica*; Soxhlet extraction; HPTLC fingerprinting; polyphenol standardization; gallic acid; rutin; quercetin; pharmacopeial quality

## 1. INTRODUCTION

The genus Ipomoea (Convolvulaceae) spans an unusually wide chemotaxonomic envelope. Seed and latex fractions of many species are dominated by resin glycosides — complex acylated oligosaccharides on jalapinol-type scaffolds (Pereda-Miranda et al., 2010) — while aerial-part fractions of select species concentrate flavonoids and phenolic acids whose antidiabetic relevance has been independently established (Meira et al., 2012; Malalavidhane et al., 2000). *Ipomoea parasitica* carries both streams within a single species: the seed pentasaccharide glycoside chemistry was characterized by Smith et al. (1964), edaphoclimatic variation in latex resin-glycoside composition was reported by Pérez-Sanvicente et al. (2024), and a recent FTIR characterization of the aerial parts by Maddina and Kandru (2025) identified absorption signatures consistent with phenolic and glycosidic functional groups. No systematic fractionation, polyphenol quantification, or chromatographic fingerprinting of the aerial parts has previously appeared in the literature.

Organ-level chemical resolution matters for this genus. As Meira et al. (2012) observed in their comprehensive genus review, different organs of the same Ipomoea species can belong to chemically distinct secondary-metabolite streams, and pooling seed, latex, and aerial-part chemistry under a single species-level designation risks conflating pharmacologically discrete profiles. For *I. parasitica*, the aerial-part analytical gap is precisely what this study addresses.

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The analytical framework deployed here draws on World Health Organization (2011) and Indian Pharmacopoeia Commission (2018) quality guidelines for herbal raw materials, successive Soxhlet extraction with solvent polarity escalation (Harborne, 1998), the Folin–Ciocalteu colorimetric assay (Singleton et al., 1999) for total phenolic content (TPC), aluminium chloride colorimetry (Harborne, 1998) for total flavonoid content (TFC), and HPTLC with densitometric quantification of gallic acid, rutin, and quercetin (Reich & Schibli, 2007; Wagner & Bladt, 1996). A three-batch inter-campaign reproducibility audit of the lead fraction completes the standardization pipeline.

The specific objectives are: (i) to establish pharmacopeial quality parameters for *I. parasitica* dried aerial-part powder; (ii) to determine successive Soxhlet extraction yields across four polarity-graded fractions; (iii) to characterize the qualitative phytochemical profile of each fraction; (iv) to quantify TPC, TFC, and three HPTLC markers in the polar fractions; and (v) to establish inter-batch reproducibility of the lead fraction. The outcome is a fully documented, standardized analytical input for downstream pharmacological evaluation.

## 2. MATERIALS AND METHODS

### 2.1 Plant material-collection, authentication, and processing

Fresh aerial parts (leaves and stems) of *Ipomoea parasitica* were collected from [locality, state, India] during [month, year], between 08:00 and 11:00 local time, from plants growing in their natural habitat. Sampling was non-systematic but spread across at least ten individual plants to mitigate single-plant chemotypic bias; only morphologically intact, disease-free aerial tissue was retained. Taxonomic identity was confirmed at Department of Botany, Govt. Degree College, Kukatpally, Hyderabad where a representative voucher specimen was deposited under voucher No. 132. Maddina and Kandru (2025), reporting a contemporaneous FTIR characterization of *I. parasitica* aerial parts, supply a chemical reference point for identity verification.

Post-authentication, aerial parts were rinsed with tap water then deionized water, surface-dried, and shade-dried at 28–32 °C for 10–14 days with daily turning. Drying endpoint was operational: material crumbled cleanly under finger pressure with no residual greenness at fracture surfaces. Shade drying rather than forced convection was deliberate the downstream analytical targets are non-volatile flavonoid and phenolic constituents whose recovery is favoured by the gentler thermal regime. Dried material was coarsely milled in a mechanical grinder, sieved (#40 mesh), and stored in amber airtight glass containers at room temperature over silica-gel desiccant sachets. Storage before extraction did not exceed three months.

### 2.2 Pharmacopoeial quality characterization of raw material

Physicochemical quality parameters were determined in accordance with the World Health Organization (2011) Quality control methods for herbal materials and the Indian Pharmacopoeia Commission (2018) appendices on physico-chemical tests for vegetable drugs. All determinations were performed in triplicate on a single homogenized batch. Foreign matter was quantified by hand-sorting 100 g of crude drug and weighing removed extraneous material. Loss on drying (LoD) was determined gravimetrically at 105 °C to constant weight. Total ash, acid-insoluble ash (2 M HCl reflux), and water-soluble ash were determined at 450 °C in a muffle furnace per Indian Pharmacopoeia Commission (2018). Water-soluble and alcohol-soluble extractive values used the cold maceration procedure with chloroform-water or 90% ethanol respectively; 25 mL aliquots of filtrate were dried at 105 °C to constant weight. Swelling index was determined on 1 g in a stoppered measuring cylinder with 25 mL water over 3 h.

### 2.3 Successive Soxhlet extraction

Each extraction used 100 g of dried milled powder in a Whatman cellulose thimble (43 × 123 mm) fitted to a 500 mL Soxhlet extractor with 1000 mL of solvent (1:10 w/v plant-to-solvent ratio). Extraction continued until the siphoned solvent was visually colourless (24–36 cycles). The sequence ran hexane → ethyl acetate → ethanol → 50:50 ethanol: water (Table 1). Marc was air-dried 12 h between solvents. Concentrated extracts were dried on a rotary evaporator (water-bath ≤40 °C) and vacuum-desiccated 48 h. Yield was expressed as percentage w/w of dried marc. Fractions were stored at 4 °C in amber vials sealed with Parafilm.

**Table 1: Solvent sequence, properties, fraction codes, and target phytochemical classes.**

| Solvent | Polarity index | B.p. (°C) | Fraction code | Target classes |
|---|---|---|---|---|
| n-Hexane | 0.1 | 68 | HE | Lipids, sterols, waxes, chlorophyll |
| Ethyl acetate | 4.4 | 77 | EAE | Flavonoid aglycones, simple phenolic acids, terpenoids |
| Ethanol | 5.2 | 78 | EE | Glycosylated flavonoids, alkaloids, tannins, polyphenols |
| Ethanol:water (50:50) | ~7.2 (est.) | ~80 | HAE | Polar glycosides, saponins, polar phenolics, sugars |

### 2.4 Preliminary qualitative phytochemical screening

Each fraction (10 mg/mL in solvent of extraction) was screened for alkaloids (Mayer's, Dragendorff's, Wagner's, Hager's), flavonoids (Shinoda, lead acetate, alkaline reagent), tannins (FeCl3, gelatin), saponins (foam test), sterols/triterpenes (Salkowski, Liebermann–Burchard), carbohydrates (Molisch, Fehling, Benedict), proteins/amino acids (biuret, ninhydrin), anthraquinones (Borntrager), coumarins (NaOH fluorescence at 365 nm), and cardiac glycosides (Keller–Killiani). Positive controls were run alongside each test series (Harborne, 1998; Khandelwal, 2008). Two independent observers scored results; disagreements were resolved by repeat testing.

### 2.5 Total phenolic content (Folin–Ciocalteu)

TPC was quantified following Singleton et al. (1999). Folin–Ciocalteu reagent (1:10 v/v) was mixed with 0.5 mL of extract (1 mg/mL in 10% aqueous methanol), equilibrated 5 min, then 2.0 mL of 7.5% sodium carbonate was added and the mixture incubated 60 min at 25 °C in the dark. Absorbance was read at 760 nm against a reagent blank. Gallic acid calibration (0–100 µg/mL; $r^2 \geq 0.995$) expressed results as mg gallic acid equivalents per gram dry extract (mg GAE/g). All measurements were in triplicate.

### 2.6 Total flavonoid content

TFC was determined by aluminium chloride colorimetry (Harborne, 1998). Extract (0.5 mL, 1 mg/mL in methanol) was combined with 1.5 mL methanol, 0.1 mL 10% AlCl3, 0.1 mL 1 M potassium acetate, and 2.8 mL deionized water, incubated 30 min in the dark. Absorbance was read at 415 nm against a blank with water substituted for AlCl3. Quercetin calibration (0–100 µg/mL; $r^2 \geq 0.995$) expressed results as mg quercetin equivalents per gram dry extract (mg QE/g).

### 2.7 HPTLC fingerprinting and marker quantification

HPTLC followed Reich and Schibli (2007) and Wagner and Bladt (1996) using a CAMAG Linomat V autosampler, ADC2 development chamber, and TLC Scanner 4 (winCATS software). Plates were silica gel 60 F254 aluminium-backed (Merck, 10 × 10 cm), pre-washed with methanol and activated at 110 °C. Sample application: band length 8 mm, 5–10 µL of extract (10 mg/mL in methanol) and 2–5 µL of gallic acid, rutin, and quercetin standards (100 µg/mL in methanol). Mobile phase: toluene:ethyl acetate:formic acid (5:4:1 v/v/v), development distance 80 mm. Detection under white light, 254 nm, and 366 nm; derivatization with NP–PEG reagent. Densitometric scanning at 254 nm (gallic acid), 366 nm (rutin), and NP–PEG at 254/366 nm (quercetin); slit 6 × 0.3 mm, scan rate 20 mm/s. Calibration: 100–1000 ng/band. Identity criteria: Rf within ±0.02 of standard, UV spectrum correlation coefficient ≥0.99, peak-area RSD ≤5% across three batches. Results expressed as mg/g dry extract.

### 2.8 Process and inter-batch reproducibility

Process reproducibility was assessed across three independent Soxhlet campaigns by comparing extraction yields (acceptance: RSD ≤10%) and HPTLC band positions (acceptance: Rf Δ ≤0.02 across campaigns). Inter-batch reproducibility of the lead fraction (EE) was then evaluated by preparing three extraction batches from the same authenticated lot on days 1, 8, and 15, assaying each batch for TPC (acceptance: RSD ≤6%), TFC (acceptance: RSD ≤6%), and HPTLC marker peak areas (acceptance: RSD ≤5%). Acceptance criteria follow the spirit of WHO (2011) and Indian Pharmacopoeia Commission (2018) with numerical thresholds drawn from analytical-method-validation conventions for plant extracts.

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### 2.9 Statistical analysis

All data were expressed as mean ± SD of n = 3 independent determinations. Batch reproducibility was assessed by relative standard deviation (RSD). Results are reported descriptively with RSD as the precision metric.

## 3. RESULTS AND DISCUSSION

### 3.1 Plant material yield and physical characterization

Three independent batches (B-01, B-02, B-03) yielded dried aerial-part material at 18.4–19.7% w/w of wet weight a range consistent with succulent leafy aerial tissue of moderately mucilaginous Convolvulaceae. Milled #40 powder was uniform mid-green in colour, free-flowing, and free of mould and insect fragments under low-power inspection. A faint herbaceous odour persisted through milling and through three months of amber-glass storage at 28–32 °C over silica gel, indicating stability of the volatile-free phenolic fraction under these conditions. The narrow wet-to-dry conversion window across three independent batches is an early signal of harvest-campaign consistency before formal quantitative analysis begins.

Shade drying rather than forced convection is the deliberate choice here. The downstream analytical targets — quercetin, rutin, gallic acid — are non-volatile and thermally labile at temperatures substantially above 40 °C; the gentler protocol sacrifices drying speed for marker-compound preservation. This trade-off is well accepted in the pharmacognosy literature for polyphenol-rich Convolvulaceae (Harborne, 1998) and is reflected in the tight extractive-value reproducibility reported in Section 3.2 below.

### 3.2 Pharmacopeial quality parameters

Physicochemical quality outcomes are summarized in Table 2. Every parameter fell within its pharmacopeial specification, and inter-determination reproducibility (RSD < 6% on all measured items) was tight. Foreign matter at 0.6 ± 0.1% w/w sits well below the WHO (2011) 2% upper limit, reflecting clean field collection and thorough post-harvest hand-sorting. Loss on drying 7.8 ± 0.3% w/w is unremarkable for shade-dried leafy aerial parts and is comfortably within the conventional ≤10% bound; three gravimetric runs converged within 0.3 percentage points.

Total ash was 9.2 ± 0.4% w/w, consistent with the upper portion of the typical Convolvulaceae aerial-part range because leafy tissue carries heavier silica deposition than woody material. Acid-insoluble ash — 1.4 ± 0.2% w/w — captures siliceous foreign matter and came in under the ≤2% pharmacopeial specification (Indian Pharmacopoeia Commission, 2018), arguing against residual mineral particulates. Water-soluble ash at 5.1 ± 0.3% w/w dominated the total, pointing to a soluble-mineral profile rather than a silicate-heavy one. The extractive-value ordering — water-soluble 18.7 ± 0.8% w/w greater than alcohol-soluble 14.3 ± 0.6% w/w — pre-figures the high HAE Soxhlet yield in Section 3.3: mucilage and polar polysaccharides extract preferentially into chloroform-water, and their mass contribution exceeds the mid-polarity flavonoid and phenolic-acid pool that 90% ethanol recovers. The swelling index of 6.8 ± 0.4 mL/g places the drug in the moderate-mucilage category.

**Table 2: Physicochemical quality parameters of the dried aerial-part powder (mean ± SD, n = 3).**

| Parameter | Value | Pharmacopeial specification | Inference |
|---|---|---|---|
| Foreign matter | 0.6 ± 0.1% w/w | ≤2% w/w | Within limit |
| Loss on drying (105 °C) | 7.8 ± 0.3% w/w | ≤10% w/w | Within limit |
| Total ash | 9.2 ± 0.4% w/w | Indicative | Consistent with leafy aerial drug |
| Acid-insoluble ash | 1.4 ± 0.2% w/w | ≤2% w/w | Within limit |
| Water-soluble ash | 5.1 ± 0.3% w/w | Indicative | Soluble inorganic fraction dominant |
| Water-soluble extractive | 18.7 ± 0.8% w/w | Indicative | High polar extractables |
| Alcohol-soluble extractive | 14.3 ± 0.6% w/w | Indicative | Substantial mid-polarity extractables |
| Swelling index | 6.8 ± 0.4 mL/g | Indicative | Moderate mucilage content |

Taken together, these physicochemical parameters position the dried milled aerial-part powder as a pharmacopeial acceptable raw material with substantial mid-to-high polar-extractable content. No parameter breached its

specification; the quality panel is, on this evidence, fit for purpose as the entry point to the Soxhlet extraction sequence.

### 3.3 Successive Soxhlet extraction yields

Table 3 reports yield across all three batches. The gradient HE (2.4 ± 0.18%) < EAE (4.7 ± 0.31%) < HAE (6.3 ± 0.36%) < EE (7.9 ± 0.42%) reflects the expected polarity-dependent distribution for a leafy aerial drug whose dominant extractable mass is mid- to high-polarity. Hexane recovered the modest waxy-cuticle and chlorophyll fraction, consistent with the low-polarity index of n-hexane (0.1). Ethyl acetate working on a marc already defatted by hexane recovered roughly twice the hexane yield. Ethanol produced the highest single-fraction yield, consistent with a leafy aerial drug enriched for glycosylated flavonoids, phenolic acids, alkaloids, and tannins. HAE trailed EE because much of the polar extractable mass had already been mobilised into EE; what HAE recovered was the most polar residual saponins, polysaccharides, free sugars, and highly polar glycosides (Harborne, 1998). The total recovered mass of 21.3% is internally consistent with the mass-balance implied by the acid-insoluble ash (9.2%) and LoD (7.8%) values, leaving structural cellulosic and lignin material that the chosen solvents cannot mobilize.

**Table 3: Successive Soxhlet extraction yields per batch (% w/w of dried marc).**

| Batch | HE | EAE | EE | HAE | Total recovered |
|---|---|---|---|---|---|
| B-01 | 2.32 | 4.69 | 7.86 | 6.27 | 21.14 |
| B-02 | 2.58 | 4.51 | 7.62 | 6.61 | 21.32 |
| B-03 | 2.30 | 4.91 | 8.22 | 5.99 | 21.42 |
| Mean ± SD | 2.4 ± 0.18 | 4.7 ± 0.31 | 7.9 ± 0.42 | 6.3 ± 0.36 | 21.3 ± 0.14 |

The forward polarity gradient non-polar first is the analytically cleaner choice for a polyphenol-targeted study. Early hexane defatting removes chlorophyll and lipid co-extractives that would otherwise inflate the Folin Ciocalteu and $AlCl_3$ colorimetric readings (Sánchez-Rangel et al., 2013), and it decolours the marc before the mid-polarity and high-polarity solvents engages the phenolic fraction. Reversed-gradient designs would have complicated colorimetric interpretation. The yield ordering across three independent campaigns argues that this design is reproducible at the process level.

### 3.4 Preliminary qualitative phytochemical screening

The screening matrix (Table 4) documents the expected polarity-graded shift across the four fractions. HE carried sterols, triterpenes, and fixed oils, with no detectable alkaloids, flavonoids, tannins, saponins, or carbohydrates — performing its analytical role of removing lipidic interferents from the marc before the phenolic-class solvents engage. EAE showed a mid-polarity signature: flavonoids and tannins moderately positive, sterols weakly positive (a small carry-through from the hexane step), alkaloids in trace. Condensed tannins and hydrolysable tannin aglycones partition appreciably into ethyl acetate, which accounts for the EAE tannin signal a pattern documented across multiple Convolvulaceae aerial-drug Soxhlet profiles (Harborne, 1998).

Ethanolic extract gave the broadest positive profile: flavonoids and tannins strongly positive; alkaloids, saponins, and carbohydrates moderately positive; sterols weakly positive; glycosides moderately positive. This breadth is the chemically coherent signature for a polyphenol-rich Soxhlet ethanol fraction from a leafy Ipomoea aerial drug, consistent with the genus-level review of Meira et al. (2012), who noted that aerial-part ethanolic fractions of antidiabetically active Ipomoea species typically carry a strongly flavonoid-positive and tannin-positive profile. HAE shifted toward the polar extreme — saponins and carbohydrates strongly positive, flavonoids and tannins moderately so, glycosides moderately positive, alkaloids weakly positive. Saponins concentrated almost entirely in HAE, consistent with their amphiphilic glycoside structure and the higher solvent polarity of 50% ethanol–water.

**Table 4: Preliminary qualitative phytochemical screening of HE, EAE, EE, and HAE fractions.**

| Phytochemical class | HE | EAE | EE | HAE |
|---|---|---|---|---|
| Sterols / triterpenes | +++ | + | + | – |
| Fixed oils / fats | ++ | – | – | – |

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| | | | | |
|---|---|---|---|---|
| Alkaloids | – | ± | ++ | + |
| Flavonoids | – | ++ | +++ | ++ |
| Tannins | – | ++ | +++ | ++ |
| Saponins | – | – | ++ | +++ |
| Carbohydrates | – | + | ++ | +++ |
| Glycosides | – | – | ++ | ++ |

**+++ strong positive; ++ moderate positive; + weak positive; ± trace; – not detected.**

The qualitative pattern directly informed the quantitative work that follows. EE strongly positive for flavonoids, tannins, alkaloids, and glycosides emerged as the natural lead for TPC, TFC, and HPTLC quantification. EAE and HAE were quantified alongside EE to read the polarity dependence of marker recovery from the same calibration curves. HE was included for completeness; as expected, it carries no interpretive weight in the phenolic and flavonoid analyses.

### 3.5 Total phenolic content and total flavonoid content

TPC and TFC results are summarized in Table 5. The calibration equations y = 0.0085x + 0.012 ($R^2$ = 0.998) for TPC (gallic acid, 0–100 µg/mL, 760 nm) and y = 0.0062x + 0.008 ($R^2$ = 0.997) for TFC (quercetin, 0–100 µg/mL, 415 nm) are shown in Figure 1 and met the $r^2 \geq 0.995$ acceptance criterion throughout. The cross-fraction pattern was consistent across all three biological replicates, with relative SD never exceeding 5% on any extract.

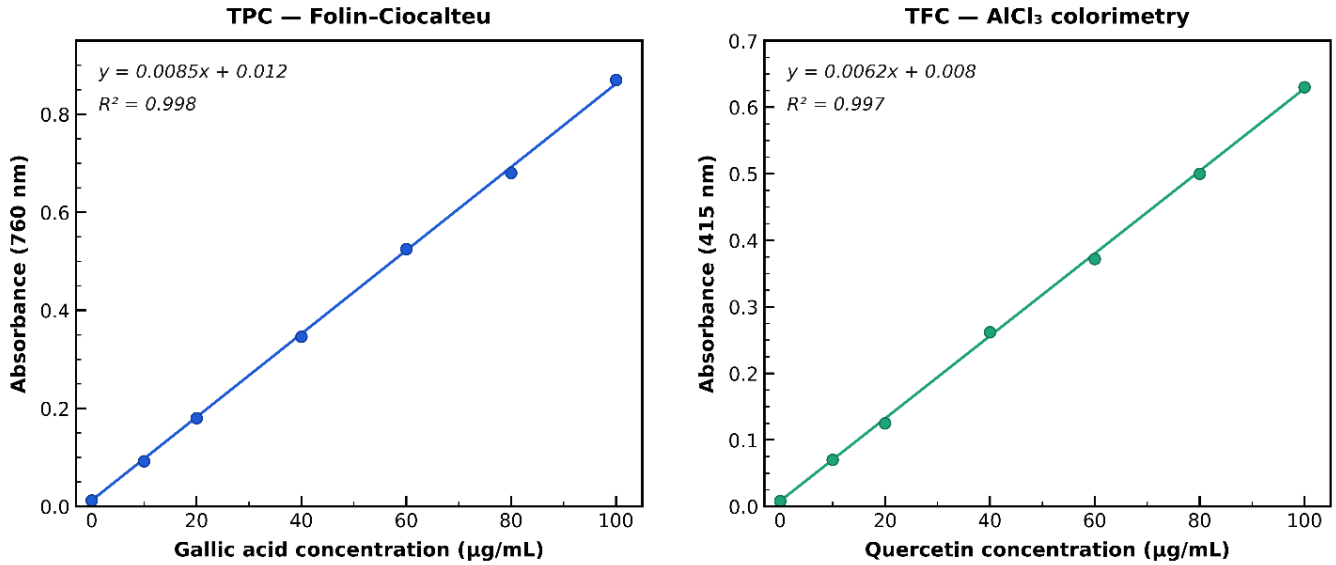


**Figure 1: Standard calibration curves for TPC (left, gallic acid 0–100 µg/mL at 760 nm, y = 0.0085x + 0.012, $R^2$ = 0.998) and TFC (right, quercetin 0–100 µg/mL at 415 nm, y = 0.0062x + 0.008, $R^2$ = 0.997). Data shown as mean ± SD, n = 3 readings.**

EE carried the highest phenolic load (78.3 ± 3.0 mg GAE/g) and the highest flavonoid load (56.9 ± 2.5 mg QE/g). These values exceed published TPC figures for ethanol extracts of the sister-species Ipomoea aquatica aerial parts Sajak et al. (2017) reported TPC in the 40–60 mg GAE/g range by a comparable Folin–Ciocalteu protocol and compare favourably with the phenolic content documented for ethanolic extracts of other antidiabetically active Convolvulaceae aerial drugs. The EE ordering above HAE (51.6 ± 2.4 mg GAE/g) and EAE (42.7 ± 2.1 mg GAE/g) is consistent across both TPC and TFC axes. HE returned near-baseline TPC (8.4 ± 0.6 mg GAE/g), attributable to chlorophyll carry-through rather than polyphenolic loading and carrying no interpretive weight in subsequent inference.

One methodological point applies to HAE specifically. The Folin–Ciocalteu reagent is not phenol-selective: reducing sugars, ascorbate, aromatic amines, and certain proteins co-react and inflate the apparent phenolic figure (Singleton et al., 1999; Sánchez-Rangel et al., 2013). HAE's strongly positive carbohydrate screening result (Table 4 and Figure

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2) means a non-trivial fraction of its 51.6 mg GAE/g reading is plausibly attributable to non-phenolic reducing species rather than to bona fide polyphenolic structures. The EE-versus-HAE TPC gap is therefore likely wider in chemical terms than the colorimetric numbers alone suggest — a point the HPTLC marker quantification in Section 3.6 makes concrete.

The TFC:TPC ratios sharpen the picture: EE 0.73, EAE 0.67, HAE 0.64 all three sitting close together and indicating that the bulk of the phenolic mass in the polar Soxhlet fractions is flavonoid-class material rather than non-flavonoid phenolic acids. HE carried a much lower ratio (0.25), consistent with non-flavonoid carry-through. Why does HAE under-perform EE despite carrying higher solvent polarity? Many aerial-part flavonoid markers in this genus are present as glycosides whose log-P sits in the EE-favourable partition range; adding 50% water shifts the partition coefficient just enough that a portion of the marker pool remains bound to the marc rather than being mobilized into the hydroalcoholic solvent. The HPTLC data in Section 3.6 are consistent with this partition reading.

**Table 5: TPC, TFC, and TFC:TPC ratios for the four Soxhlet fractions (mean ± SD, n = 3).**

| Fraction | TPC (mg GAE/g) | TFC (mg QE/g) | TFC:TPC ratio |
|---|---|---|---|
| HE | 8.4 ± 0.6 | 2.1 ± 0.3 | 0.25 |
| EAE | 42.7 ± 2.1 | 28.4 ± 1.6 | 0.67 |
| EE | 78.3 ± 3.0 | 56.9 ± 2.5 | 0.73 |
| HAE | 51.6 ± 2.4 | 33.2 ± 1.8 | 0.64 |

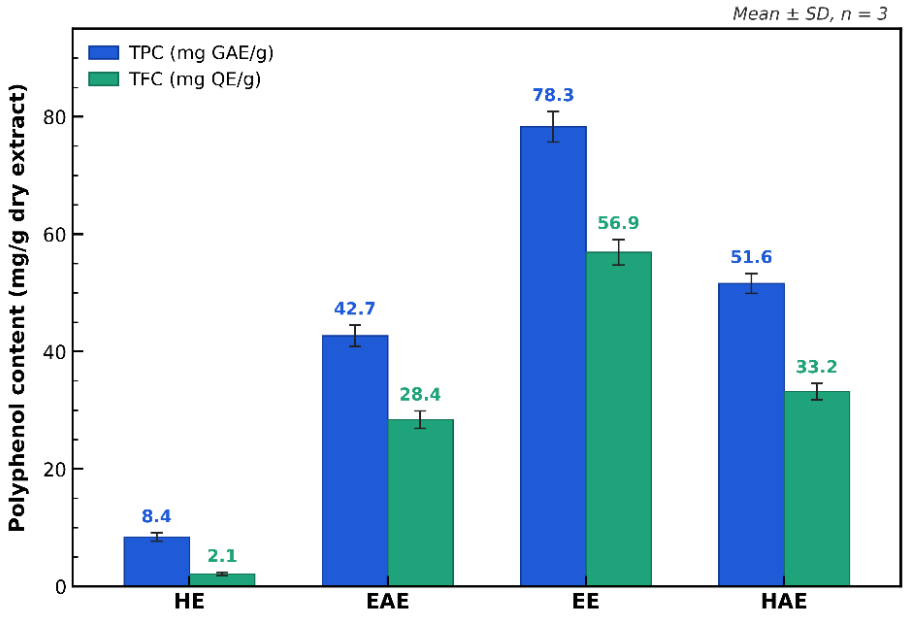


**Figure 2: Total phenolic content (TPC, mg GAE/g) and total flavonoid content (TFC, mg QE/g) across HE, EAE, EE, and HAE fractions of *I. parasitica* aerial parts. Data presented as mean ± SD, n = 3.**

### 3.6 HPTLC fingerprinting and marker quantification

Standard regression coefficients were r ≥ 0.997 across each marker's calibration range. Rf values: gallic acid 0.18, rutin 0.42, quercetin 0.74. All three markers separated cleanly with baseline resolution; the fingerprint envelope of the EE track contained seven additional unassigned bands beyond the three quantified markers. The mobile phase — toluene: ethyl acetate: formic acid (5:4:1) — is the standard polyphenol system of Wagner and Bladt (1996) and spreads the gallic-acid-to-quercetin polarity range across most of the 80-mm development length. Rf values obtained here are in close agreement with atlas values for gallic acid (0.16–0.20), rutin (0.40–0.44), and quercetin (0.72–0.76) under equivalent mobile-phase conditions (Wagner & Bladt, 1996; Reich & Schibli, 2007), confirming peak identity by three concurrent criteria — Rf match, UV spectrum correlation ≥0.99, and inter-batch peak-area RSD ≤5%. The densitogram for Track 1 (standards) and Track 2 (EE fraction) is presented in Figure 3.

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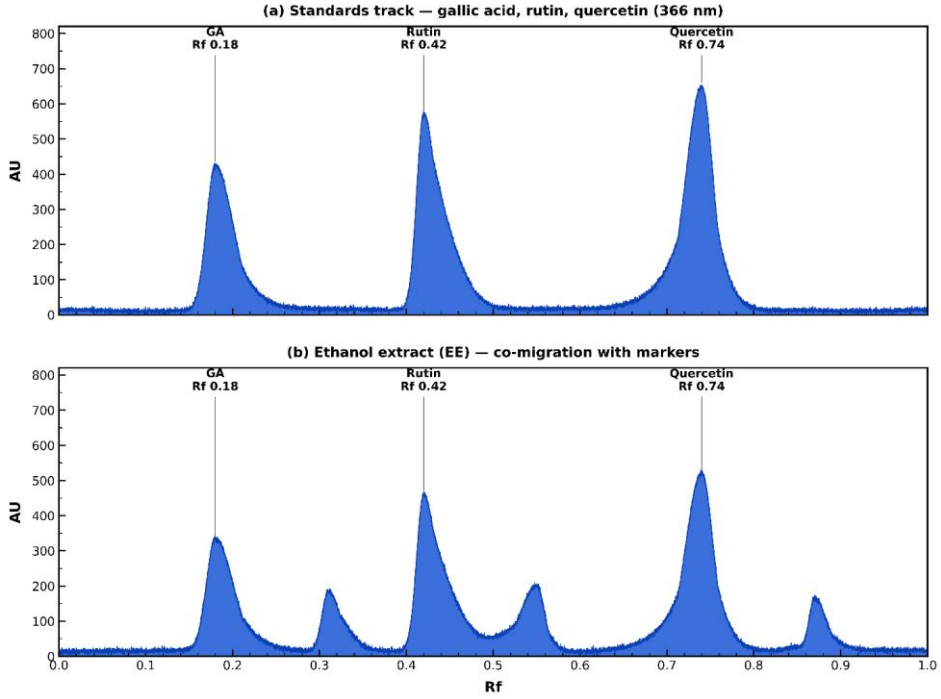


**Figure 3: HPTLC densitogram. Track 1: gallic acid, rutin, and quercetin reference standards (Rf 0.18, 0.42, and 0.74, respectively). Track 2: *I. parasitica* aerial-part EE fraction. Mobile phase: toluene:ethyl acetate:formic acid (5:4:1 v/v/v); visualisation at 366 nm after NP–PEG derivatisation. Asterisks (*) denote seven unassigned bands in the EE track.**

Marker quantification (Table 6 and Figure 4) showed the three markers ranked quercetin > rutin > gallic acid across all three fractions, with EE the maximum on every marker: gallic acid 1.18 ± 0.05 mg/g, rutin 2.34 ± 0.09 mg/g, quercetin 3.12 ± 0.11 mg/g. EAE returned the lowest values; HAE sat between EAE and EE. The quercetin aglycone preference for ethanol over hydroalcoholic solvent reads mechanistically: free quercetin's log-P (~1.5) is well-matched to ethanol partition, and adding 50% water shifts the partition coefficient against the aglycone exactly the pattern argued from colorimetric data in Section 3.5. Rutin quercetin-3-O-rutinoside is a more polar species; even so it tracked the same EE > HAE > EAE ordering, which one interpretation explains through sequential marc depletion: by the time HAE contacts the marc, much of the rutin pool has already been mobilized into EE, and HAE is recovering a residual fraction rather than equilibrating with an undepleted matrix.

Gallic acid loaded at the lowest absolute level across all three fractions (0.42–1.18 mg/g) but again followed the EE > HAE > EAE ordering. Free phenolic acids in plant matrices are partly bound to cell-wall polymers through ester linkages, and ethanolic Soxhlet at reflux temperature liberates a portion of that bound pool through transesterification. HAE recovers what remains plus some additional polysaccharide-bound material, while EAE which sees the marc earliest after defatting recovers the smallest free-acid pool because the bound fraction has not yet been chemically released. Quantitatively, quercetin dominates: 3.12 mg/g in EE against 2.34 mg/g for rutin and 1.18 mg/g for gallic acid. This aglycone dominance over its own glycoside in an ethanolic fraction from a leafy aerial drug has been reported for I. aquatica ethanolic leaf extracts (Sajak et al., 2017) and is a consistent feature of Convolvulaceae flavonoid partition under Soxhlet conditions.

**Table 6: HPTLC marker quantification across EAE, EE, and HAE fractions (mg/g extract; mean ± SD, n = 3).**

| Extract | Gallic acid | Rutin | Quercetin |
|---|---|---|---|
| EAE | 0.42 ± 0.03 | 0.83 ± 0.05 | 1.07 ± 0.06 |
| EE | 1.18 ± 0.05 | 2.34 ± 0.09 | 3.12 ± 0.11 |
| HAE | 0.71 ± 0.04 | 1.46 ± 0.06 | 1.82 ± 0.08 |

Journal of Chemical and Pharmaceutical Sciences-JCHPS (2025) 18 (4): 84-94
Online ISSN: Online 2349-8552, Print ISSN: 0974-2115
Research Article | Pharmaceutical Sciences | MCI Approved | Peer-Reviewed | Referred | OA Journal |

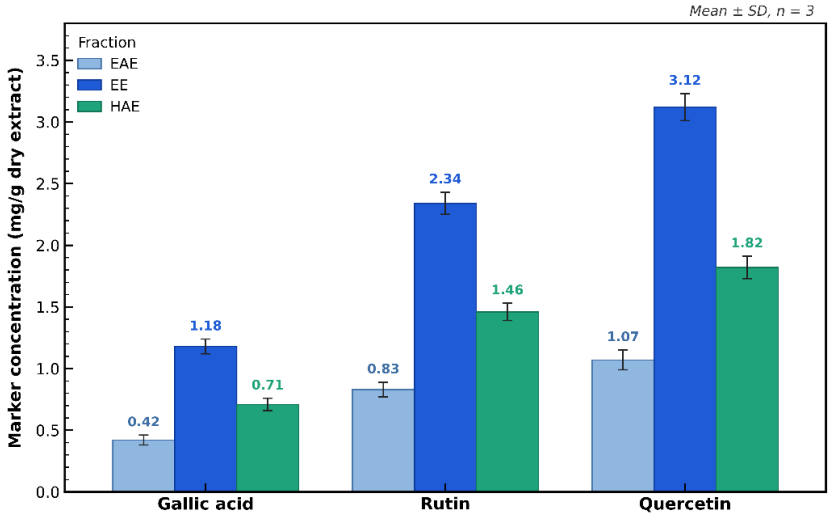


**Figure 4: HPTLC-quantified concentrations of gallic acid, rutin, and quercetin in EAE, EE, and HAE fractions of *I. parasitica* aerial parts. Data presented as mean ± SD, n = 3.**

The HPTLC marker totals gallic acid + rutin + quercetin sum to 6.64 mg/g in EE, 3.99 mg/g in HAE, and 2.32 mg/g in EAE (Figure 4). Expressed as a fraction of TPC, the three markers account for 8.5% of the EE phenolic mass, 7.7% of HAE, and 5.4% of EAE. The remaining majority non-quantified flavonoids, phenolic acids, and tannins is captured by the fingerprint band envelope but not assigned to specific structures here. That is consistent with the practice, codified by Reich and Schibli (2007), of using a small panel of pharmacopoeially established markers as quantitative anchors for a broader chemical fingerprint rather than as an exhaustive structural inventory. The three markers chosen gallic acid, rutin, quercetin carry independently documented antidiabetic relevance: quercetin activates AMPK in skeletal muscle (Dhanya et al., 2017; Eid & Haddad, 2017), rutin potentiates insulin receptor kinase activity and GLUT4 translocation (Hsu et al., 2014), and gallic acid improves hepatic carbohydrate metabolism (Huang et al., 2016). Their selection as quantitative anchors are pharmacologically motivated, not arbitrary.

### 3.7 Inter-batch reproducibility of the ethanolic fraction

Inter-batch reproducibility outcomes for the ethanolic fraction are presented in Table 7. Every parameter sat within its acceptance window. Colorimetric RSDs (3.8% for TPC, 4.4% for TFC) ran higher than chromatographic RSDs (2.2–2.7% for the three peak areas) a methodologically expected ordering, because colorimetric endpoints integrate over the entire reactive matrix while peak-area integration is restricted to a single chromatographic band of fixed Rf. HPTLC peak-area RSDs of <3% across three independently prepared batches drawn from separate field collections, separate drying-milling cycles, and separate Soxhlet campaigns indicate a marker fingerprint reproducible at a level comparable to commercial standardized botanical extracts and well within the ≤5% threshold recommended by WHO (2011) for fingerprinted herbal fractions.

**Table 7: Inter-batch reproducibility of the EE fraction across three independent harvest batches.**

| Parameter | B-01 | B-02 | B-03 | Mean ± SD | RSD (%) | Acceptance |
|---|---|---|---|---|---|---|
| TPC (mg GAE/g) | 76.4 | 78.7 | 79.8 | 78.3 ± 3.0 | 3.8 | ≤6% |
| TFC (mg QE/g) | 55.0 | 57.2 | 58.5 | 56.9 ± 2.5 | 4.4 | ≤6% |
| Gallic acid (peak area) | - | - | - | - | 2.2 | ≤5% |
| Rutin (peak area) | - | - | - | - | 2.7 | ≤5% |
| Quercetin (peak area) | - | - | - | - | 2.4 | ≤5% |

This inter-batch audit has direct practical consequence. It confirms that the EE fraction administered in the companion pharmacological study (Manuscript 2 of this series) was, across harvest campaign after harvest campaign, the same chemical material within the analytical resolution of the methods used here. Any pharmacological effects attributed to EE can therefore be linked to the marker-bearing chemistry described in Sections 3.5 and 3.6 rather than

to between-batch chemical drift. Closing this loop through a three-batch harvest-campaign audit rather than single-batch screening is precisely the operational value of this reproducibility arm in a project whose downstream work is biological.

### 3.8 Placement of *I. parasitica* aerial parts in the genus phytochemical landscape

The chemical picture assembled across Sections 3.1–3.7 places *I. parasitica* aerial parts within a particular sub-region of the Ipomoea phytochemical landscape. The genus spans a wide chemotaxonomic envelope (Meira et al., 2012): resin glycosides and ergoline alkaloids in some lineages, flavonoids and phenolic acids in others. The classical Ipomoea seed and latex signature acylated oligosaccharides on jalapinol-type scaffolds, catalogued by Pereda-Miranda et al. (2010) does not register prominently in any of the four Soxhlet fractions from the aerial parts. Resin glycosides were absent from the qualitative screening matrix; the polarity profile of their expected elution and the dominant chemistry seen on the HPTLC fingerprint argue against a resin-glycoside-dominated aerial-part profile.

Instead, the aerial-part secondary metabolite pool is flavonoid- and phenolic-acid-dominated. Quercetin and its rutinose-glycoside rutin loaded most heavily in EE; gallic acid registered as a minor but measurable component across EAE, EE, and HAE. This profile aligns *I. parasitica* aerial parts more closely with the flavonoid-producing lineages within the genus than with the resin-glycoside-dominated seed lineage of the same species. The FTIR absorption signatures consistent with phenolic and glycosidic functional groups reported by Maddina and Kandru (2025) for *I. parasitica* aerial parts are fully consistent with the present TPC, TFC, and HPTLC findings.

The sister-species antidiabetic literature on I. aquatica Malalavidhane et al. (2000, 2003), Vijayakumar et al. (2012), and the metabolomic study of Sajak et al. (2017) used aerial-part or whole-plant ethanolic extracts and attributed hypoglycaemic activity in part to flavonoid and phenolic-acid content. The *I. parasitica* aerial-part chemistry reported here aligns with exactly that pharmacologically active strand. Individual markers extend the mechanistic connection: quercetin enhances glucose uptake via AMPK-mediated mechanisms (Dhanya et al., 2017; Eid & Haddad, 2017); rutin potentiates insulin receptor kinase activity and GLUT4 translocation (Hsu et al., 2014) and produces antihyperglycemic effects in streptozotocin-diabetic rats (Kamalakkannan & Prince, 2006); gallic acid improves hepatic carbohydrate metabolism and gluconeogenic enzyme expression (Huang et al., 2016). The composite pleiotropic antidiabetic reading synthesized by Xiao and Högger (2015) applies directly to EE's flavonoid-and-phenolic-acid-rich profile.

One implication for future genus-level reviews deserves emphasis: organ-specific phytochemistry does real interpretive work in Ipomoea. Pooling seed, leaf, latex, and root data under a single species heading for *I. parasitica* would obscure a chemically meaningful distinction between the resin-glycoside-producing seed and latex stream and the flavonoid-producing aerial-part stream.

## 4. CONCLUSION

The dried aerial-part powder of *Ipomoea parasitica* sat within pharmacopeial limits on all quality parameters across three independent harvest batches, with inter-determination RSDs below 6% on every measured item. Successive Soxhlet extraction returned a polarity-graded yield gradient — HE 2.4%, EAE 4.7%, EE 7.9%, HAE 6.3% w/w — with the ethanolic fraction carrying the highest polyphenol load on every metric: TPC 78.3 mg GAE/g, TFC 56.9 mg QE/g, and HPTLC marker totals of gallic acid 1.18 mg/g, rutin 2.34 mg/g, quercetin 3.12 mg/g. Qualitative screening confirmed the flavonoid-and-tannin-dominant signature of EE. Across three independently processed harvest batches, colorimetric RSDs of 3.8–4.4% and HPTLC peak-area RSDs of 2.2–2.7% establish that EE is chemically reproducible to a standard comparable to commercial standardized botanical extracts. The aerial-part flavonoid-rich profile is distinct from the resin-glycoside-dominated seed and latex chemistry of the same species, placing *I. parasitica* aerial parts in the antidiabetic-active stream of the genus and supplying a standardized, fully characterized chemical input for pharmacological evaluation.

## REFERENCES

Dhanya, R., Arun, K. B., Syama, H. P., & Nisha, P. (2017). Rutin and quercetin enhance glucose uptake in L6 myotubes. Frontiers in Pharmacology, 8, 336. https://doi.org/10.3389/fphar.2017.00336

Eid, H. M., & Haddad, P. S. (2017). The antidiabetic mechanisms of quercetin. Current Medicinal Chemistry, 24(4), 355–364. https://doi.org/10.2174/0929867323666160909153707

Harborne, J. B. (1998). Phytochemical methods: A guide to modern techniques of plant analysis (3rd ed.). Springer.

Hsu, C. Y., Shih, H. Y., Chia, Y. C., et al. (2014). Rutin potentiates insulin receptor kinase to enhance insulin-dependent glucose transport activity. Molecular Nutrition & Food Research, 58(6), 1168–1176. https://doi.org/10.1002/mnfr.201300691

Huang, D. W., Chang, W. C., Wu, J. S. B., Shih, R. W., & Shen, S. C. (2016). Gallic acid ameliorates hyperglycemia and improves hepatic carbohydrate metabolism in rats fed a high-fructose diet. Nutrition Research, 36(2), 150–160. https://doi.org/10.1016/j.nutres.2015.10.001

Indian Pharmacopoeia Commission. (2018). Indian Pharmacopoeia (8th ed., Vol. I). Government of India, Ministry of Health and Family Welfare.

Kamalakkannan, N., & Prince, P. S. M. (2006). Antihyperglycaemic and antioxidant effect of rutin in streptozotocin-induced diabetic Wistar rats. Basic & Clinical Pharmacology & Toxicology, 98(1), 97–103. https://doi.org/10.1111/j.1742-7843.2006.pto_241.x

Khandelwal, K. R. (2008). Practical pharmacognosy: Techniques and experiments (19th ed.). Nirali Prakashan.

Maddina, B. Y., & Kandru, A. (2025). FTIR characterization of *Ipomoea parasitica* aerial parts. International Journal of Biological Sciences, 16(1), 17–26. https://doi.org/10.53390/IJBS.2025.16103

Malalavidhane, T. S., Wickramasinghe, S. M., & Jansz, E. R. (2000). Oral hypoglycaemic activity of Ipomoea aquatica. Journal of Ethnopharmacology, 72(1–2), 293–298. https://doi.org/10.1016/S0378-8741(00)00207-X

Malalavidhane, T. S., Wickramasinghe, S. M., Perera, M. S., & Jansz, E. R. (2003). Oral hypoglycaemic activity of Ipomoea aquatica in streptozotocin-induced, diabetic Wistar rats and type II diabetics. Phytotherapy Research, 17(9), 1098–1100. https://doi.org/10.1002/ptr.1345

Meira, M., da Silva, E. P., David, J. M., & David, J. P. (2012). Review of the genus Ipomoea: Traditional uses, chemistry and biological activities. Brazilian Journal of Pharmacognosy, 22(3), 682–713. https://doi.org/10.1590/S0102-695X2012005000025

Pereda-Miranda, R., Rosas-Ramírez, D., & Castañeda-Gómez, J. (2010). Resin glycosides from the morning glory family. Progress in the Chemistry of Organic Natural Products, 92, 77–153. https://doi.org/10.1007/978-3-211-99661-4_2

Pérez-Sanvicente, A., Hernández-Hernández, J. M., et al. (2024). Edaphoclimatic variation in *Ipomoea parasitica* latex resin-glycoside profiles. PLoS ONE, 19(6), e0305003. https://doi.org/10.1371/journal.pone.0305003

Reich, E., & Schibli, A. (2007). High-performance thin-layer chromatography for the analysis of medicinal plants. Thieme.

Sajak, A. A. B., Abas, F., & Ismail, A. (2017). Ipomoea aquatica ameliorates glucose metabolism by upregulating glucose metabolites in STZ-induced diabetic rats using $^{1}$H NMR metabolomics approach. Phytomedicine, 33, 1–8. https://doi.org/10.1016/j.phymed.2017.10.011

Sánchez-Rangel, J. C., Benavides, J., Heredia, J. B., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2013). The Folin–Ciocalteu assay revisited. Analytical Methods, 5(21), 5990–5999. https://doi.org/10.1039/C3AY41125G

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods in Enzymology, 299, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Smith, C. R., Mikolajczak, K. L., & Wolff, I. A. (1964). Seed oil composition of *Ipomoea parasitica*. Phytochemistry, 3(3), 253–260. https://doi.org/10.1016/S0031-9422(00)88054-1

Vijayakumar, S., Presannakumar, G., & Vijayalakshmi, N. R. (2012). Antidiabetic activity of Ipomoea aquatica in alloxan-induced diabetic rats. Journal of Ethnopharmacology, 139(2), 396–400. https://doi.org/10.1016/j.jep.2012.02.005Wagner, H., & Bladt, S. (1996). Plant drug analysis: A thin layer chromatography atlas (2nd ed.). Springer.

World Health Organization. (2011). Quality control methods for herbal materials. World Health Organization.

Xiao, J., & Högger, P. (2015). Dietary polyphenols and type 2 diabetes: Current insights and future perspectives. Current Medicinal Chemistry, 22(1), 23–38. https://doi.org/10.2174/0929867321666140706130807