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A Comprehensive Review of Analytical Methods Used for Phytochemical Studies in the Genus *Impatiens* L.

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Abstract: *The genus Impatiens L. (Balsaminaceae) comprises approximately 1,200 species distributed primarily across tropical and subtropical hotspots, including the Himalayas and Western Ghats. While several species, such as I. balsamina and I. walleriana, are recognised for their ornamental value and pharmacological potential—notably their antimicrobial, anti-inflammatory, and anti-rheumatic properties—a significant portion of the genus remains phytochemically uncharacterized. This review provides a comprehensive overview of the analytical landscape, tracing the evolution from traditional screening to advanced instrumental techniques for studying the genus's complex secondary metabolites, including naphthoquinones, flavonoids, and triterpenoid saponins. The transition from conventional extraction methods, such as Soxhlet and maceration, to high-efficiency techniques, including ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and pressurised liquid extraction (PLE), is evaluated. These modern methods significantly reduce solvent consumption and protect thermolabile compounds. The role of chromatographic techniques is examined, highlighting the shift from qualitative HPTLC fingerprinting to high-resolution quantification via RP-HPLC. Furthermore, the indispensable nature of hyphenated platforms, specifically LC-MS and GC-MS, in achieving definitive structural elucidation of complex glycosides and volatile profiles is discussed. Finally, this review addresses persistent challenges—such as the lack of reference standards and inherent chemical variability—and advocates for the adoption of UPLC-HRMS-based metabolomics to standardise herbal products and further explore the chemical diversity of this botanically rich genus..*

Keywords: analytical methods, *Impatiens*, Western Ghats

1. Introduction

The genus *Impatiens* L. was established by the Swedish botanist Linnaeus in the 1750s and, together with *Hydrocera* Bl., constitutes the family Balsaminaceae (Linnaeus, 1753). There are approximately 1200 recorded species of this genus worldwide, which occur mainly in tropical and subtropical regions (Grey-Wilson, 1980). Members of this genus are distributed across five diverse hotspots: Madagascar, the tropical African continent, southern India and Sri Lanka, the eastern Himalayas, southwestern China, and the broader Southeast Asia region (Stevens, 2001). They are also reported from Japan, the northern zone of Europe, Russia and North America (Yu et al., 2016).

In India, *Impatiens* species are mainly found in the Himalayas and the Western Ghats, with a disjunctive distribution of more than 130 species in each hotspot (Bhaskar, 2012). There are about 280 species of *Impatiens* recorded so far in India, of which 162 species are endemic, and about 106 species occur in the Western Ghats, showing 90% of endemism (Bhaskar, 2012; Vivekananthan, 1997).

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The *Impatiens* species occur from sea level to 4000 m altitude and often grow in forest margins, in valleys, roadside troughs, and along streams, generally on humid soils (Vrchotova et al., 2011). Some species in the genus, *I. balsamina* L. and *I. walleriana* Hook. f. in particular, have pharmaceutical importance. Furthermore, some taxa are planted as ornamentals. However, due to such cultivations outside the respective home ranges, some species have become invasive pests, e.g. *I. capensis* Meerb., I. parviflora DC., and *I. glandulifera* Royle (Coakley & Petti, 2021). On the other hand, some species have become endangered in their native ranges, and 32 *Impatiens* members currently in the Red List as threatened plants (IUCN, 2024).

Many species of *Impatiens* have been cornerstones in traditional medicine systems. *I. balsamina* L. is traditionally used for its anti-rheumatic, anti-inflammatory, and antimicrobial properties, particularly for treating fungal and bacterial skin infections (Ding et al., 2008) whereas *I. capensis* Meerb. is ethnobotanically valued for treating dermatological conditions, including poison ivy rash (Motz et al., 2015). The pharmacological importance of these species can be linked to the presence of a wide range of secondary metabolites reported from the *Impatiens* genus, including naphthoquinones, triterpenoids, saponins, flavonoids, anthocyanins, phenolic acids, coumarins etc. (Sakunphueak & Panichayupakaranant, 2010). These metabolites exhibit pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory and cytotoxic activities, as well as allelopathic properties (Delgado-Rodríguez et al., 2023; Sakunphueak & Panichayupakaranant, 2012).

The *Impatiens* genus shows a high level of chemical complexity and biological diversity, including variations in chemotypes, genetics, and environmental factors. Many *Impatiens* species have not yet been studied for their phytochemical properties. This review describes how analytical methods for studying the phytochemistry of *Impatiens* have developed over time and explains the current best practices, providing a valuable resource for researchers in natural product chemistry and pharmacognosy.

Sample Preparation

The reliability and efficiency of phytochemical analysis depend upon the sample preparation. As *Impatiens* metabolites span a wide range of polarities, sample preparation and extraction methods are crucial for obtaining reliable results.

Conventional Extraction Techniques:

Historically, Maceration and Soxhlet extraction methods using a single solvent or a mixture of solvents with increasing polarity (e.g., hexane, ethyl acetate, methanol, water) have been widely used. Soxhlet extraction with ethanol remains a standard method for obtaining broad-spectrum extracts from *I. balsamina*. However, these methods are often time-consuming, require large volumes of solvent, and can lead to thermal degradation of labile compounds, such as anthocyanins and specific naphthoquinone precursors (Delgado-Rodríguez et al., 2023)

Modern and Green Extraction Techniques:

To improve efficiency and minimise environmental impact, several modern extraction and purification techniques have been increasingly adopted in the study of *Impatiens* species.

Ultrasound-assisted extraction (UAE) employs acoustic cavitation to disrupt plant cell walls, thereby enhancing solvent penetration into the matrix. This results in significantly reduced extraction time and lower solvent consumption compared with conventional maceration or Soxhlet extraction. UAE has been successfully applied to recover polyphenols from various *Impatiens* species with high yield and reproducibility (Vieira et al., 2016).

Microwave-assisted extraction (MAE) utilises microwave energy to rapidly and selectively heat the solvent–sample mixture, accelerating the partitioning of target analytes into the extraction solvent. Although less commonly reported in *Impatiens* than in the UAE, MAE is gaining traction for the extraction of heat-stable secondary metabolites, such as certain flavonoids and anthocyanins (Guo et al., 2022).

Pressurised Liquid Extraction (PLE), also known as accelerated solvent extraction (ASE), operates at elevated temperature, typically 50–200 °C and pressure 10–20 MPa, enabling rapid and exhaustive extraction with minimal

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solvent volumes. This technique is particularly valuable for comprehensive metabolite profiling of *Impatiens* tissues prior to liquid chromatography–mass spectrometry (LC-MS) analysis, offering high recovery of both polar and medium-polarity compounds (Rahmanian et al., 2015).

For the isolation and purification of individual marker compounds from complex *Impatiens* extracts, High-Speed Counter-Current Chromatography (HSCCC) has proven crucial. HSCCC, coupled with high-performance liquid chromatography (HPLC-HSCCC), enables preparative-scale separation of flavonoids and other phenolic constituents without solid stationary phases, thereby preventing sample loss and irreversible adsorption (Huang et al., 2019). This approach has been effectively employed, for example, in the purification of kaempferol glycosides from *I. glandulifera* flowers (Chen et al., 2009).

The final step prior to instrumental analysis typically involves sample clean-up or fractionation, most commonly using solid-phase extraction (SPE). SPE effectively removes interfering matrix components such as lipids, chlorophylls, and sugars, reduces ion suppression/enhancement effects, and improves chromatographic resolution and detection sensitivity, particularly in highly sensitive LC-MS workflows (Nguyen et al., 2025).

Non-Chromatographic Analytical Methods

Before using advanced instruments, researchers often use simple, quick, and affordable non-chromatographic tests for initial phytochemical screening and bulk measurement.

Qualitative Screening:

Traditional chemical tests help to identify whether major compound groups are present in *Impatiens* extracts. Qualitative phytochemical analysis of plant extracts uses chemical tests to determine the presence or absence of different classes of compounds, such as alkaloids, tannins, flavonoids, saponins, and terpenoids. These simple, cost-effective tests, such as the Wagner test for tannins or the Biuret test for proteins, provide a preliminary screening to identify potential bioactive constituents that give a plant its medicinal properties (Adil et al., 2024).

Quantitative Assays:

Quantitative phytochemical analysis of *Impatiens* extracts involves spectrophotometric assays for key compounds like Total Phenolic Content (TPC) (Folin-Ciocalteu method), Total Flavonoid Content (TFC) (Aluminum Chloride method), and Total Alkaloid Content (TAC), alongside qualitative tests (e.g., Keller-Killiani for glycosides, Borntrager's for anthraquinones, FeCl3 for tannins) and antioxidant assays (like DPPH) to measure bioactive potential, often using solvents like methanol or ethyl acetate for extraction (Dagne et al., 2025).

Chromatographic Separation and Quantification

Chromatography is the backbone of phytochemical analysis in *Impatiens*, enabling the separation, identification, and quantification of individual compounds in complex mixtures.

Thin Layer Chromatography (TLC) and High-Performance TLC (HPTLC):

TLC and its advanced version, HPTLC, are indispensable for rapid qualitative profiling, purity checking, and semi-quantitative analysis. TLC/HPTLC offers a versatile platform for fingerprinting different *Impatiens* species, which is essential for chemotaxonomic studies and quality control. HPTLC has been successfully applied to quantify characteristic naphthoquinones, such as lawsone and 2-MNQ, in various *Impatiens* extracts. The simultaneous determination of multiple naphthoquinones provides a key marker for plant authentication. Using specific spray reagents (e.g., Natural Product-Polyethylene Glycol reagent), flavonoids can be visualised under UV light, yielding characteristic chromatograms (fingerprints) that confirm the presence and approximate concentration of these compounds (Dagne et al., 2025).

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High-Performance Liquid Chromatography (HPLC):
HPLC, particularly Reversed-Phase (RP-HPLC), is the most widely utilised technique for the quantitative and qualitative analysis of non-volatile and semi-volatile secondary metabolites in *Impatiens*. Its high resolution, speed, and reproducibility make it the method of choice for quality assurance.

Gas Chromatography (GC):
GC is primarily reserved for the analysis of volatile and semi-volatile compounds, most notably the essential oils derived from some *Impatiens* species such as terpenoids, monoterpenes, sesquiterpenes, and occasionally, derivatised less-volatile compounds (Jakubska-Busse et al., 2023a).

Hyphenated Techniques: Structural Elucidation and Profiling
The non-specific nature of UV detection in HPLC often limits definitive structural confirmation. The integration of powerful separation techniques with mass spectrometry (MS) has revolutionised the phytochemical analysis of *Impatiens*, providing the necessary structural information.

Liquid Chromatography-Mass Spectrometry (LC-MS):
LC-MS is the gold standard for comprehensive chemical profiling, enabling rapid identification of both known and potentially novel compounds. In an LC-MS workflow, the initial separation by HPLC is followed immediately by mass detection. This allows researchers to determine molecular weight and structural confirmation. LC-MS has been essential for characterising the highly complex glycosidic structures abundant in *Impatiens*, such as flavonoid glycosides and triterpenoid Saponins (Chua, 2016).

Gas Chromatography-Mass Spectrometry (GC-MS):
GC-MS combines the excellent separation power of GC with the definitive identification capability of MS. It is beneficial for identifying volatile compounds and is often used in preliminary non-targeted screening of semi-volatile extracts. Extracts from *I. minor* and *I. balsamina*, particularly those prepared with non-polar solvents (petroleum ether, chloroform, ethyl acetate), have been subjected to GC-MS to identify compounds such as fatty acids, sterols, and certain terpenoids, including oleanene derivatives. Compounds separated by GC are ionized, and their resultant mass spectra are compared against vast spectral libraries (e.g., NIST), allowing for rapid tentative identification of several dozen compounds in a single run (Jakubska-Busse et al., 2023b).

Challenges and Future Perspectives
Despite the advancements, the phytochemical analysis of *Impatiens* still faces several hurdles:
Lack of Reference Standards: The immense species diversity, over 1000 species, means that many unique or minor metabolites lack commercial reference standards, complicating routine quantitative analysis and requiring laborious isolation work.
Complexity of Glycosides: Many bioactive compounds in *Impatiens* exist as complex glycosides (e.g., anthocyanins with multiple sugar moieties), whose isomeric forms are difficult to resolve and identify without HRMS technology.
Method Variability: Differences in geographical location, harvesting time, storage, and processing such as drying technique, as seen in *I. hawkeri* analysis, can dramatically alter the chemical profile, necessitating rigorous validation for method transferability.
Thermal Stability: The characteristic naphthoquinones can be thermally unstable or reactive, demanding mild extraction and separation conditions to prevent artifacts (Sukumaran et al., 2025).

2. Conclusion

The pharmacological richness of the *Impatiens* genus, driven by compounds like naphthoquinones and complex flavonoids, continues to demand sophisticated analytical techniques. The field has progressed from classical screening and TLC to highly selective and sensitive HPLC methods for targeted quantification. Today, the integration of LC-MS and GC-MS stands as the analytical benchmark, offering unparalleled capabilities for structural elucidation and comprehensive chemical profiling. As research shifts toward understanding the genus's immense chemical diversity and standardising herbal products, the adoption of UPLC − HRMS-based metabolomics platforms, coupled with powerful chemometrics, is essential. Future research must prioritise the development of universally applicable, validated analytical protocols that account for the genetic and environmental variability inherent in this medicinally and ornamentally important plant genus.

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