International Journal of Research in Pharmacology & Pharmacotherapeutics (IJRPP)
IJRPP | Vol.15 | Issue 1 | Jan - Mar -2026
www.ijrpp.com
ISSN: 2278-2648 DOI : https://doi.org/10.61096/ijrpp.v15.iss1.2026.155-161

Extraction Strategies and Chromatographic Approaches for Phytochemical Evaluation and Standardization of *Aegle marmelos* (L.) Correa: A Review

Dr. Kannan. S*, Dr.Lakshmi Devi. S, Pranav. H, Priyadharshini. M, Priyadharshini Bai. M, Prakash. A, Muthuselvi. A

Department of Pharmacognosy, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore - 641044, Tamil Nadu, India

*Corresponding Author: Dr. S. Kannan
Email: kannanonline2@gmail.com

Published by: 13.02.2026

Abstract *Aegle marmelos* (L.) Correa, commonly known as Bael, is an important medicinal plant extensively used in traditional systems of medicine. The therapeutic efficacy of *Aegle marmelos* is attributed to the presence of diverse phytoconstituents such as coumarins, alkaloids, flavonoids, tannins, and phenolic compounds. Scientific validation and standardization of herbal drugs derived from *Aegle. marmelos* require optimized extraction techniques and reliable analytical methods. This review article summarizes the extraction procedures and chromatographic techniques, with special emphasis on thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), and high-performance liquid chromatography (HPLC), employed for phytochemical evaluation of *Aegle. marmelos*. Comparative aspects of conventional and advanced extraction methods are discussed along with their influence on phytochemical yield and analytical performance. The role of chromatographic fingerprinting and marker-based quantification in quality control and standardization of *Aegle marmelos based* herbal formulations is highlighted.

Keywords: *Aegle marmelos*, extraction methods, HPTLC, HPLC, phytochemical analysis, standardization

1. Introduction

Aegle marmelos (L.) Correa belongs to the family Rutaceae and is indigenous to the Indian subcontinent. The plant has been traditionally used in Ayurveda, Siddha, and Unani systems of medicine for the treatment of gastrointestinal disorders, diabetes mellitus, inflammatory conditions, and liver ailments (1). Almost all parts of the plant, including leaves, fruits, bark, roots, and seeds, possess medicinal properties.

Phytochemical investigations have reported that *Aegle marmelos* contains a wide range of bioactive constituents such as coumarins, alkaloids, flavonoids, terpenoids, tannins, and phenolic compounds (2). Among these, marmelosin (imperatorin), umbelliferon, scopoletin, and aegeline are considered important marker compounds due to their pharmacological significance (3).

Among these, alkaloids such as aegeline and its derivatives are considered significant due to their reported biological activities. However, quantitative analysis of these alkaloids is challenging because of their low abundance in leaf material and the complexity of crude extracts. Hence, the development of accurate, sensitive, and validated analytical methods is essential for reliable estimation of these marker compounds.

Aegle marmelos is an important ingredient of several Ayurvedic formulations, including Dashmularishta, Bilvadivati, Bilvadi churna, and Bilva taila. Proper standardization of these formulations requires validated analytical procedures capable of consistent quantification of bioactive markers. Although conventional extraction methods such as Soxhlet extraction and maceration, as well as modern techniques like ultrasonication, are commonly employed, systematic comparison of their efficiency in extracting alkaloids from *Aegle marmelos* leaves has been limited. Furthermore, extensive quantitative HPLC profiling of alkaloid markers in the leaves has not been comprehensively reported.

Therefore, the present study aims to develop and validate a simple, accurate, and robust HPLC method for the separation and quantification of alkaloid marker compounds in methanolic extracts of *Aegle marmelos* leaves. In addition, the study evaluates the effect of different extraction methods on alkaloid content and establishes an HPLC fingerprint suitable for routine analysis and quality control of *Aegle marmelos* leaf material (6).

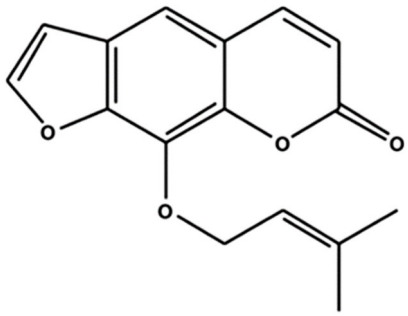
2. Phytochemical Constituents of *Aegle marmelos*

Several classes of secondary metabolites have been reported from *Aegle marmelos*. The major phytochemical groups include coumarins such as marmelosin (imperatorin), umbelliferon, scopoletin, marmin, and psoralen; alkaloids such as aegeline and aegelenine; flavonoids; and phenolic compounds (4). Marmelosin is widely used as a chemical marker due to its abundance and therapeutic relevance.

3. Reported Extraction Techniques for Aegle marmelos

3.1 Conventional Extraction Methods

Maceration and Soxhlet extraction are commonly employed conventional techniques for extraction of phytoconstituents from *Aegle marmelos*. Maceration involves soaking the powdered plant material in solvents such as methanol or ethanol at room temperature for prolonged periods, whereas Soxhlet extraction provides continuous hot extraction, resulting in higher recovery of alkaloids and coumarins (5).



Structure of marmelosin

Fig 1. Chemical structure of marmelosin (imperator in), a major coumarin marker compound of *Aegle marmelos.*

3.2 Advanced Extraction Methods

Ultrasonic-assisted extraction has been explored to reduce extraction time and enhance mass transfer. Comparative studies revealed that Soxhlet extraction yielded higher concentrations of alkaloids compared to sonication, while sonication offered the advantage of reduced processing time (6).

Table 1. Comparison of extraction methods used for *Aegle marmelos*. (Karmase A, Prasanna K, RasabattulaS,BhutaniKK. Quantification and comparison of extraction methods for alkaloids in *Aegle marmelos* leaves by HPLC.Natural Product Communications. 2014;9(7):981–983)

Extraction method	Solvent used	Plant part	Obser-vations
Maceration	Methanol /Ethanol	Leaves	Moderate yield
Soxhlet extraction	Methanol	Leaves	Higher alkaloid yield
Ultra sonication	Methanol	Leaves	Reduced extractio n time

4. Analytical Techniques for Phytochemical Evaluation

4.1 Thin Layer Chromatography

Thin layer chromatography (TLC) is widely employed as a preliminary qualitative

technique for phytochemical screening and identity verification of medicinal plants. In *Aegle marmelos*, TLC has been extensively used to detect phenolic compounds, coumarins, and alkaloids prior to advanced instrumental analysis (7). The simplicity, cost-effectiveness, and minimal sample preparation requirements make TLC suitable for routine screening in herbal research laboratories.

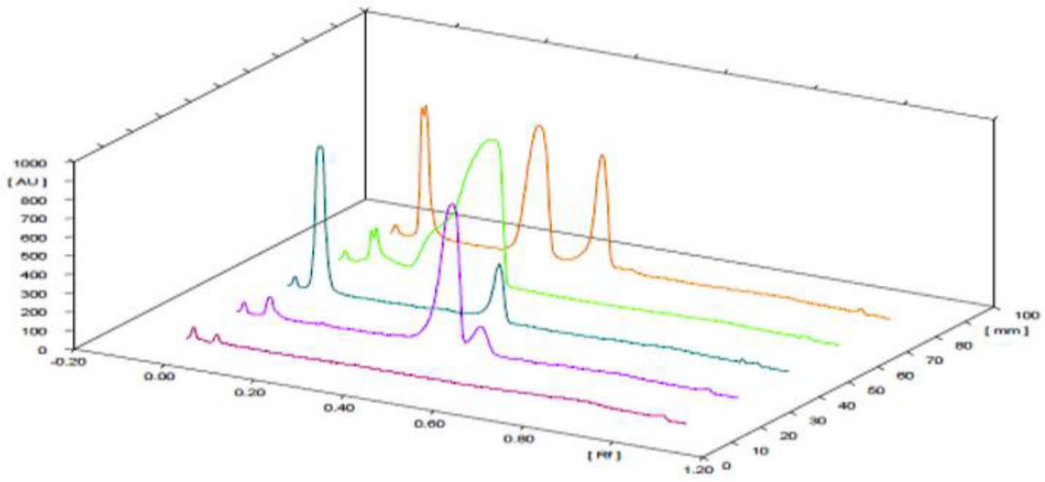
Several studies have reported the use of silica gel G plates with solvent systems comprising toluene, ethyl acetate, methanol, and formic acid for effective separation of *Aegle marmelos* phytoconstituents (8). TLC profiling of leaf and fruit extracts consistently revealed multiple resolved spots under UV illumination at 254 nm and 366 nm, indicating the presence of chemically diverse secondary metabolites. Although TLC lacks quantitative precision, its role in rapid authentication and preliminary quality assessment remains significant (9).

4.2 High Performance Thin Layer Chromatography (HPTLC)

High performance thin layer chromatography (HPTLC) represents a significant advancement over conventional TLC, offering enhanced resolution, sensitivity, and reproducibility. It is particularly valuable for fingerprint profiling of herbal drugs, where therapeutic activity is attributed to a complex mixture of constituents rather than a single compound (10).

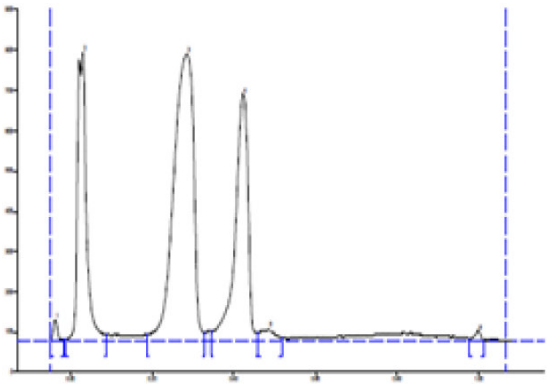
HPTLC fingerprinting of *Aegle marmelos* leaf extracts has demonstrated characteristic band patterns at specific Rf values corresponding to alkaloids, phenolics, and coumarins (11). Visualization under UV 254 nm and 366 nm revealed differential distribution of phytoconstituents, confirming the suitability of HPTLC for qualitative standardization. The three-dimensional overlay chromatograms further strengthen the reliability of HPTLC by enabling simultaneous comparison of multiple tracks and detection wavelengths (12).

Compared to HPLC, HPTLC requires smaller solvent volumes, allows parallel analysis of multiple samples, and provides visual chemical fingerprints, making it highly suitable for routine quality control and authentication of *Aegle marmelos* based herbal formulations (13).

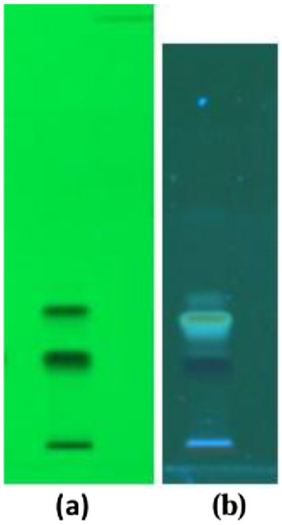


3D Overlay of HPTLC chromatogram of all tracts, at all wavelengths

Fig 2. HPTLC fingerprint profile of hydro alcoholic extract of *Aegle marmelos* leaves visualized at 254 nm and 366 nm, showing the presence of multiple phytoconstituents. (Adapted from Rathod and Share, 2024).



HPTLC chromatogram



HPTLC chromatograms visualized under a. UV 254 nm, and b. UV 366 nm

Table 2. HPTLC fingerprint characteristics of *Aegle marmelos* leaf extract.

Detection wavelength	Major Rf values	Phytoconstituents
254 nm	0.03, 0.29, 0.42	Alkaloids, phenolic
366 nm	0.49, 0.65	Coumarins

4.3 High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) is regarded as the most reliable and widely accepted analytical technique for quantitative estimation of phytochemicals. In *Aegle marmelos*, reversed-phase HPLC methods employing C18 columns have been successfully developed for the simultaneous estimation of key marker compounds such as marmelosin, umbelliferone, and scopoletin (14).

Reported HPLC methods demonstrated excellent chromatographic resolution, linearity, precision, and sensitivity, with detection wavelengths ranging from 254 to 310 nm (15). Representative chromatograms revealed well-resolved peaks with reproducible retention times, confirming the robustness of these methods for routine analysis. HPLC-based quantification is particularly important for marker-based standardization, regulatory compliance, and formulation development (16).

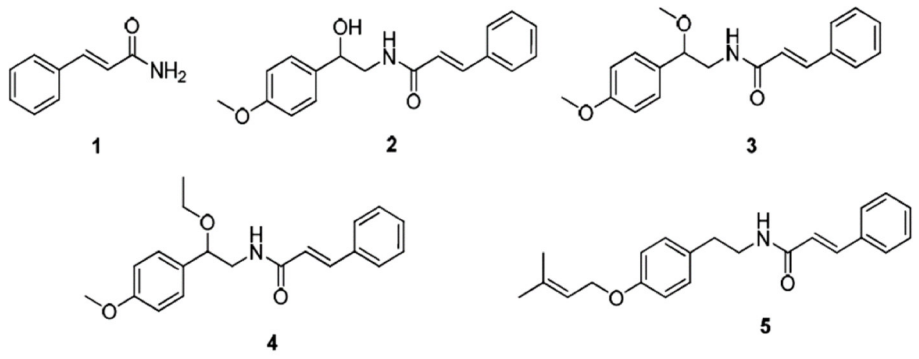


Figure 3. Representative RP-HPLC chromatogram showing separation of major marker compounds of *Aegle marmelos*, including marmelosin and related coumarins. (Adapted from Shailajan et al., 2014; Karmase et al., 2014).

Table 3. HPLC analytical conditions reported for *Aegle marmelos*.

Column	Mobile phase	Detection wavelength	Application
C18	Methanol: Water	254–310 nm	Marker quantification

5. Role of Chromatographic Fingerprinting in Standardization

Chromatographic fingerprinting has emerged as a cornerstone in the standardization of herbal medicines, particularly for plants like *Aegle marmelos*, where therapeutic activity is mediated by multiple constituents (17).Chromatographic fingerprinting using HPTLC and HPLC provides reproducible chemical profiles essential for authentication, detection of adulteration, and batch-to-batch consistency of herbal drugs. Marker-based standardization using marmelosin and aegeline is recommended for quality control of *Aegle marmelos* formulations (9). Fingerprinting approaches using HPTLC and HPLC provide comprehensive chemical profiles that reflect the intrinsic complexity of plant materials.

HPTLC fingerprinting offers visual representation of phytochemical diversity and is highly effective for routine authentication. The presence or absence of characteristic bands at defined Rf values serves as a diagnostic criterion for identity verification, while comparison across batches enables monitoring of raw material consistency (18).

HPLC fingerprinting, on the other hand, provides higher resolution and quantitative precision. Marker-based HPLC profiles facilitate accurate assessment of active constituent levels, enabling detection of substandard or adulterated samples. The combined use of HPTLC and HPLC thus offers a complementary strategy for comprehensive quality control of *Aegle marmelos*–based formulations (19).

6. Discussion

The reviewed studies demonstrate that extraction efficiency and analytical accuracy in *Aegle marmelos* are strongly influenced by the choice of extraction technique and analytical method. Conventional methods such as Soxhlet extraction remain effective for exhaustive recovery of phytoconstituents, particularly alkaloids and coumarins, although prolonged extraction time and thermal exposure may lead to degradation of thermolabile compounds. In contrast, ultrasonic-assisted and microwave-assisted extraction techniques offer reduced processing time and improved energy efficiency, although variability in extraction yield has been reported depending on solvent polarity and target phytochemical class (20, 22).

From an analytical perspective, TLC serves as a rapid and economical preliminary screening tool but lacks quantitative reliability. HPTLC provides reproducible fingerprint profiles and is particularly suitable for routine authentication and batch-to-batch consistency evaluation in herbal industries. HPLC, however,

offers superiors sensitivity, resolution, and quantitative accuracy, making it indispensable for marker-based standardisation and regulatory acceptance of herbal formulations (14, 15).

The selection of marmelosin and aegeline as marker compounds is justified by their abundance, pharmacological relevance, and consistent detectability across different plant parts and formulations. Nevertheless, variability in phytochemical content due to geographical origin, seasonal variation, and processing conditions remains a significant challenge. Future standardization strategies should integrate chromatographic techniques with chemo metric analysis and multi-marker approaches to enhance robustness and reproducibility.

7. Conclusion

Standardization of *Aegle marmelos* requires scientifically validated extraction procedures combined with reliable analytical techniques. Chromatographic methods such as HPTLC and HPLC play complementary roles in fingerprinting and quantification of marker compounds. Adoption of these approaches will enhance the quality, safety, and reproducibility of herbal formulations derived from *Aegle marmelos*.

References

1. Kushawaha H, Singhai AK. Aegle marmelos: a medicinal plant from a traditional Indian perspective. Afr J Biomed Res. 2024; 27(4):15308–15316.
2. Monika S, Thirumal M. Phytochemical and biological review of *Aegle marmelos* Linn. Future Sci OA. 2023; 9(3):FSO849.
3. Shinde PB, Katekhaye SD, Mulik MB, Laddha KS. Rapid simultaneous determination of marmelosin, umbelliferone and scopoletin from *Aegle marmelos* fruit by RP-HPLC. J Food Sci Technol. 2014; 51(9):2251–2255.
4. Mujeeb F, Khan AF, Bajpai P, Pathak N. Phytochemical study of *Aegle marmelos*: chromatographic elucidation of polyphenolics. Pharmacogn Mag. 2017; 13(Suppl 4):S791–S800.
5. Rathod S, Sihare M. Phytochemical standardization of *Aegle marmelos* leaves extract by HPTLC fingerprinting. J Adv Zool. 2024; 45(2):801–806.
6. Karmase A, Prasanna K, Rasabattula S, Bhutani KK. Quantification and comparison of extraction methods for alkaloids in *Aegle marmelos* leaves by HPLC. Nat Prod Commun. 2014; 9(7):981–983.
7. Singh N, Kumar S. TLC and HPLC fingerprint development of *Aegle marmelos* Corr and its polyherbal marketed formulations. Int J Pharm Sci Res. 2015; 6(1):162–165.
8. Shailajan S, Hande H, Joshi H, Tiwari B, Menon S. HPTLC method for evaluation of marmelosin from *Aegle marmelos* extract. J Planar Chromatogr. 2014; 27(1):23–28.
9. Meena AK, Raju I, Singh R, et al. Comparative chromatographic profiling of *Aegle marmelos* using HPLC, GC–MS and LC–MS. Phytomed Plus. 2022; 2(1):100210.
10. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd ed. Berlin: Springer; 2001.
11. Reich E, Schibli A. High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants. Stuttgart: Thieme; 2007.
12. Sethi PD. HPTLC: Quantitative Analysis of Pharmaceutical Formulations. New Delhi: CBS Publishers; 1996.
13. WHO. Quality Control Methods for Herbal Materials. Geneva: World Health Organization; 2011.
14. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Hoboken: Wiley; 2010.
15. Ong ES. Extraction methods and chemical standardization of botanicals and herbal preparations. J Chromatogram B. 2004; 812(1–2):23–33.
16. Mukherjee PK. Quality Control of Herbal Drugs. 2nd ed. New Delhi: Business Horizons; 2007.
17. Liang YZ, Xie P, Chan K. Quality control of herbal medicines. J Chromatogram B. 2004; 812(1–2):53–70.
18. Srivastava A, Gupta MM. HPLC and HPTLC methods for herbal drug standardization. Phytochemical Anal. 2012; 23(1):1–15.
19. Fan XH, Cheng YY, Ye ZL, Lin RC, Qian ZZ. Multiple chromatographic fingerprinting and chemo metrics. J Chromatogr A. 2006; 1112(1–2):171–180.
20. Azmir J, Zaidul ISM, Rahman MM, et al. Techniques for extraction of bioactive

compounds from plant materials. J Food Eng. 2013; 117(4):426–436.
21. Mandal V, Mohan Y, Hemalatha S. Microwave-assisted extraction. Pharmacogn Rev. 2007; 1(1):7–18.
22. Chemat F, Rombaut N, Sicaire AG, Meullemiestre A, Fabiano-Tixier AS, Abert-Vian M. Ultrasound assisted extraction of foodnatural products. Mechanisms, techniques, combinations, protocols and applications. Ultason Sonochem. 2017; 34:540-560.