JOURNAL OF PHARMA INSIGHTS AND RESEARCH ISSN NO. 3048-5428

RESEARCH ARTICLE

Chromatographic Isolation and Spectroscopic Characterization of Flavonoid Bioactives from *Moringa oleifera* Lam

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Publication history: Received on 30th November 2025; Revised on 11th January 2026; Accepted on 15th January 2026

Article DOI: 10.69613/b6sp6a49

Abstract: *Moringa oleifera* Lam., widely recognized as the miracle tree, represents a significant source of high-value secondary metabolites with profound pharmacological implications. The leaves of this species contain an intricate matrix of phytochemicals, including alkaloids, saponins, tannins, and specifically, a rich profile of polyphenolic flavonoids. Systematic extraction using 96% ethanol facilitates the recovery of these bioactive constituents, which are subsequently subjected to qualitative screening to verify the presence of diverse chemical classes. Separation through silica gel column chromatography using an n-butanol, acetic acid, and water solvent system yields distinct fractions, among which specific isolates show high concentrations of flavonoid compounds. Thin-layer chromatography serves as a critical tool for monitoring the resolution of these compounds, with retardation factor values aligning closely with established standards for flavonol derivatives. Spectroscopic Studies via Ultraviolet-Visible and Fourier Transform Infrared techniques showed characteristic absorption maxima and functional group vibrations, such as phenolic hydroxyl stretching and conjugated carbonyl signals, consistent with the structural architecture of quercetin. Quantitative estimations indicate varying levels of flavonoid density across isolated fractions, with peak purity levels reaching approximately 77.99% relative to standard benchmarks. The presence of these metabolites explains the robust antioxidant, anti-inflammatory, and hepatoprotective properties attributed to the plant. These results provide a scientific basis for the utilization of *Moringa oleifera* in the formulation of nutraceuticals and functional food ingredients.

Keywords: *Moringa oleifera*; Flavonoids; Isolation; Column Chromatography; Quercetin; Spectroscopic Characterization.

1. Introduction

The global interest in plant-derived bioactives has intensified due to the search for natural alternatives to synthetic therapeutic agents. *Moringa oleifera*, a fast-growing deciduous tree belonging to the Moringaceae family, stands at the forefront of this botanical research [1]. Known for its exceptional resilience in arid climates, this plant serves as a vital nutritional and medicinal resource in tropical and subtropical regions. Every morphological part of the tree, ranging from the roots to the seeds, possesses unique chemical signatures that contribute to its "miracle tree" status in ethnomedicine [2]. *Moringa oleifera* is characterized by its tripinnate leaves, fragrant white flowers, and elongated pods. Its historical use spans across various traditional systems of medicine, where it is utilized to manage malnutrition, inflammatory conditions, and microbial infections [3].

The species is indigenous to the Himalayan foothills but has become naturalized across Africa, Southeast Asia, and South America. Traditional practitioners leverage the leaves for their galactagogue, diuretic, and analgesic properties [4]. In rural communities, the leaves are often consumed as a dense nutrient source to combat anemia and vitamin deficiencies, reflecting a transition from traditional forage to a functional food [5]. The leaves of *Moringa oleifera* act as a concentrated reservoir of primary and secondary metabolites. Scientific investigations have documented significant concentrations of essential amino acids, vitamins A, C, and E, and minerals such as calcium and potassium [6]. However, the pharmacological efficacy of the plant is primarily driven by its secondary metabolic profile. The chemical landscape of the leaves includes alkaloids, phenolics, saponins, glycosides, steroids, and terpenoids [7]. Alkaloids found in the plant contribute to its potential neuroprotective effects, while saponins and tannins play roles in cholesterol management and antimicrobial defense. These compounds work in synergy, though the polyphenolic fraction remains the most researched due to its high bioavailability and safety profile [8].

Among the diverse phytochemicals, flavonoids represent the most significant class of polyphenols in *Moringa oleifera*. These low-molecular-weight phenolic compounds are categorized based on their heterocyclic core structure into flavonols, flavones,

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isoflavones, and anthocyanins [9]. The leaves are particularly rich in flavonols, specifically quercetin and kaempferol, often occurring as glycosidic derivatives. The antioxidant capacity of these flavonoids is derived from their ability to neutralize reactive oxygen species through hydrogen atom donation or electron transfer [10]. This mechanism prevents lipid peroxidation and protects cellular DNA from oxidative damage, which is a precursor to many chronic degenerative diseases [11]. Apart from antioxidant activity, flavonoids in *Moringa oleifera* exhibit potent anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines and modulating enzyme activities such as cyclooxygenase and lipoxygenase [12]. Their role extends to cardiovascular protection, where they enhance vasodilation and inhibit platelet aggregation, and to hepatoprotection, where they assist in the detoxification processes of the liver [13]. While the general benefits of *Moringa oleifera* are well-documented, the isolation of specific flavonoid constituents is required to link individual molecules to observed biological outcomes [14]. The isolation process involves sophisticated chromatographic techniques to separate complex mixtures into pure fractions. This study involves the extraction, separation, and structural elucidation of major flavonoids from the leaves to validate their therapeutic potential and provide a rigorous scientific foundation for their application in the pharmaceutical and nutraceutical industries [15, 16].

2. Experimental Methods

2.1. Sample Collection and Preparation

Fresh leaves of *Moringa oleifera* were harvested from local botanical sources. To ensure the integrity of heat-sensitive phytochemicals, the leaves were meticulously washed with deionized water to eliminate extraneous particulate matter and subsequently shade-dried at a controlled ambient temperature. Once a constant weight was achieved, the desiccated leaves were pulverized into a coarse powder using a mechanical grinder. The resulting botanical material was stored in hermetically sealed containers at room temperature to prevent oxidative degradation prior to the extraction phase.

2.2. Extraction

The extraction was conducted to maximize the yield of polyphenolic constituents while maintaining their structural stability.

2.2.1. Maceration Process

A quantitative mass of 500 g of the leaf powder was transferred into a glass vessel and immersed in 1500 mL of 96% analytical-grade ethanol. The mixture was subjected to a 72-hour maceration period (3 × 24 hours) at room temperature. Periodic mechanical agitation was employed to optimize the concentration gradient, ensuring efficient solvent penetration and the diffusion of solutes from the plant matrix into the liquid phase.

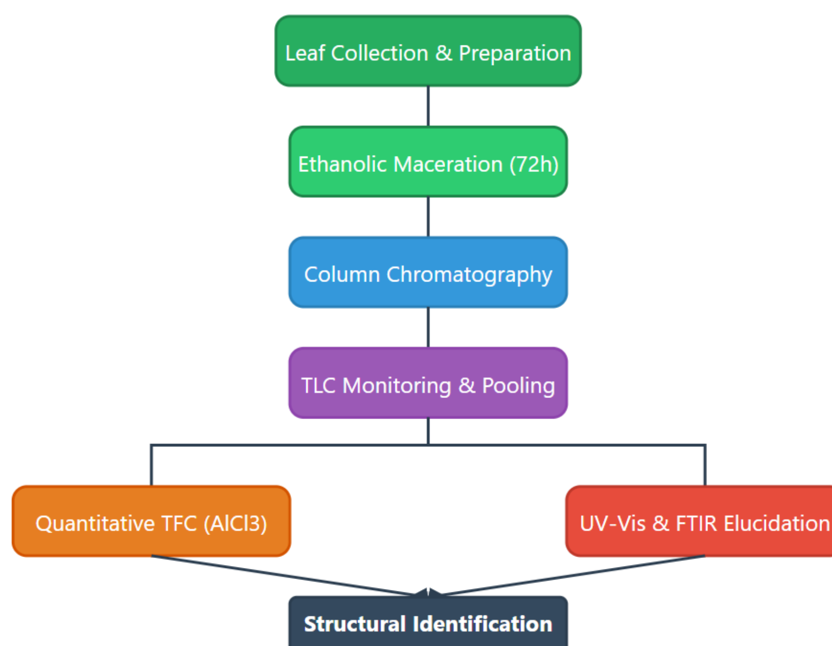

Figure 1. Flavonoid Isolation and Structural Elucidation

2.2.2. Concentration of Extract

Following maceration, the suspension was filtered through a multi-layered muslin cloth followed by Whatman No. 1 filter paper. The resulting filtrate was concentrated using a rotary vacuum evaporator at 40°C. This process yielded a viscous, dark-green crude ethanolic extract. The extract was further dried in a vacuum desiccator to eliminate residual solvent traces and was subsequently refrigerated at 4°C for downstream analysis.

2.3. Isolation and Purification of Flavonoids

The isolation strategy utilized a combination of column chromatography (CC) for bulk separation and thin-layer chromatography (TLC) for fraction monitoring and qualitative assessment [17].

2.3.1. Column Chromatography (CC)

Silica gel 60 (0.063–0.200 mm) was utilized as the stationary phase. The silica gel was activated at 105°C for 60 minutes before being slurry-packed into a glass column using the initial mobile phase. The solvent system comprised n-butanol, acetic acid, and water in a volumetric ratio of 4:1:5. After equilibration, 1 g of the crude extract, pre-adsorbed onto silica gel, was loaded onto the column bed. Elution was performed at a constant flow rate of 1 mL/min. Sequential fractions were collected and monitored based on their organoleptic properties and chromatographic behavior.

2.3.2. Thin-Layer Chromatography (TLC)

To verify the presence of flavonoids and assess the purity of the collected fractions, TLC was performed on pre-coated silica gel 60 GF254 plates. Aliquots from each fraction were spotted and developed in a saturated chamber using the n-butanol:acetic acid:water (4:1:5) system. The plates were visualized under UV radiation at 254 nm and 366 nm. For specific flavonoid detection, the plates were exposed to ammonia vapor. The Retardation factor (Rf) was calculated to compare the mobility of the isolates against established literature values.

2.4. Structural Characterization

The most promising isolate, designated as Isolate 5, was subjected to rigorous spectroscopic analysis to elucidate its functional architecture [18].

2.4.1. Ultraviolet-Visible (UV-Vis) Spectroscopy

The UV-Vis absorption profile was recorded using a double-beam spectrophotometer. The isolate was dissolved in spectroscopic-grade methanol, and the spectrum was scanned from 200 nm to 800 nm to identify characteristic absorption bands (Band I and Band II) typical of the flavonoid skeleton.

2.4.2. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR was conducted to identify the functional groups. The sample was prepared using the KBr pellet technique and scanned in the mid-infrared region (4000–400 cm^{-1}). The resulting interferogram provided data on molecular vibrations, including stretching and bending modes of the core flavonoid structure.

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3. Results and Discussion

3.1. Chromatographic Resolution of Flavonoids

The systematic fractionation of 1 g of the ethanolic extract via column chromatography yielded 12 distinct fractions. Qualitative analysis through TLC indicated that six of these fractions (Fractions 1–6) contained constituents that responded positively to flavonoid-specific reagents.

Under UV light at 366 nm, the positive fractions exhibited characteristic light blue fluorescence, which intensified upon exposure to ammonia vapor. This fluorescence is indicative of the highly conjugated systems found in flavonols. The calculated Rf value for the dominant spot in these fractions was 0.56. This value shows high congruency with documented Rf values for flavonoid standards (approximately 0.54) in similar solvent systems, suggesting that the isolated compounds belong to the flavonol subclass.

3.2. Quantitative Estimation of Total Flavonoid Content (TFC)

The TFC was determined using the aluminum chloride ($AlCl_3$) colorimetric assay, which relies on the formation of stable complexes between the aluminum ion and the C-4 keto and C-3 or C-5 hydroxyl groups of flavonoids. The addition of $AlCl_3$ resulted in a distinct bathochromic shift, producing a yellow-colored complex. Analysis of the six positive fractions revealed significant variance in flavonoid concentration. Fraction 5 exhibited the highest TFC at 17.98 mg QE/g, followed by Fraction 6 at 13.32 mg QE/g. Other fractions showed lower values, ranging from 0.10 to 7.33 mg QE/g. The elevated concentration in Fraction 5 indicates that the selected mobile phase was highly effective in selectively eluting and concentrating the primary flavonoid bioactives of *Moringa oleifera*.

Table 3. Total Flavonoid Content (TFC) of Isolated Ethanolic Fractions

Fraction Number	Total Flavonoid Content (mg QE/g extract)
Fraction 1	0.10
Fraction 2	6.35
Fraction 3	7.33
Fraction 4	3.86
Fraction 5	**17.98**
Fraction 6	13.32

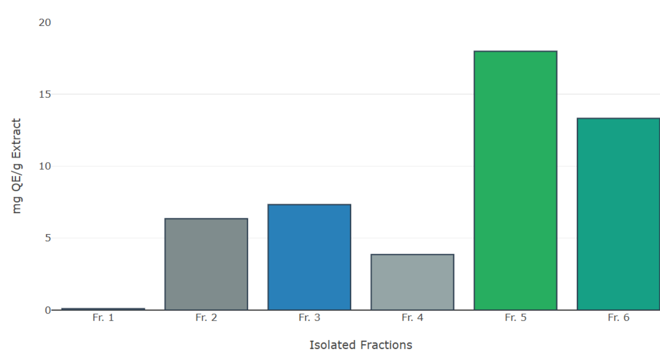

Figure 2. Distribution of Total Flavonoid Content in Chromatographic Fractions

3.3. Spectroscopic Elucidation of Isolate 5

Isolate 5 was prioritized for structural analysis due to its superior purity and high flavonoid density.

3.3.1. UV-Vis Spectral Interpretation

The UV-Vis spectrum of Isolate 5 displayed two primary absorption maxima (λmax). The first peak at 274.0 nm (Band II) corresponds to the π → π* transition within the benzoyl system (A-ring). A secondary absorption at 653.0 nm was observed, which, while unusually high for simple flavonoids, suggests potential extended conjugation or the presence of complex flavonoid-metal or flavonoid-pigment associations within the isolate. The signal at 274 nm remains a definitive marker for the aromatic A-ring of the flavonoid core.

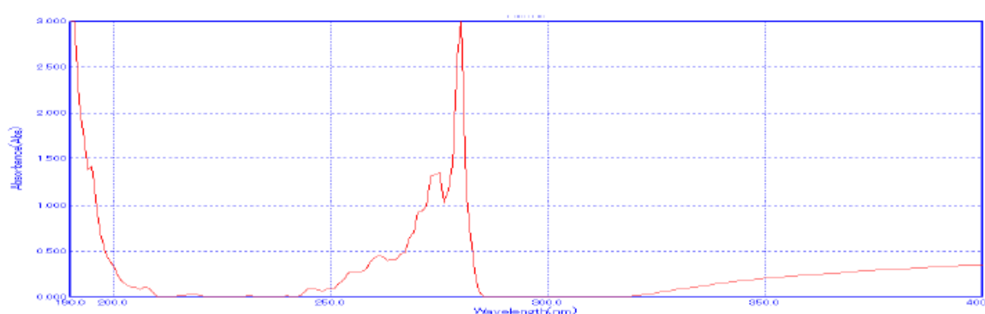

Figure 3. UV-Vis spectrum of Isolate 5 showing two primary absorption maxima (λmax) at 274.0 nm and 653.0 nm

3.3.2. FTIR Analysis

The FTIR spectrum provided critical evidence for the chemical identity of the isolate. A broad, intense absorption band at 3349.14 cm^{-1} confirmed the presence of phenolic O–H stretching vibrations. The signal at 1716.36 cm^{-1} is attributed to the conjugated carbonyl (C=O) stretching, a hallmark of the flavonol C-ring. Aromatic C=C stretching vibrations were identified at 1651.89 cm^{-1}, further validating the polycyclic aromatic nature of the compound.

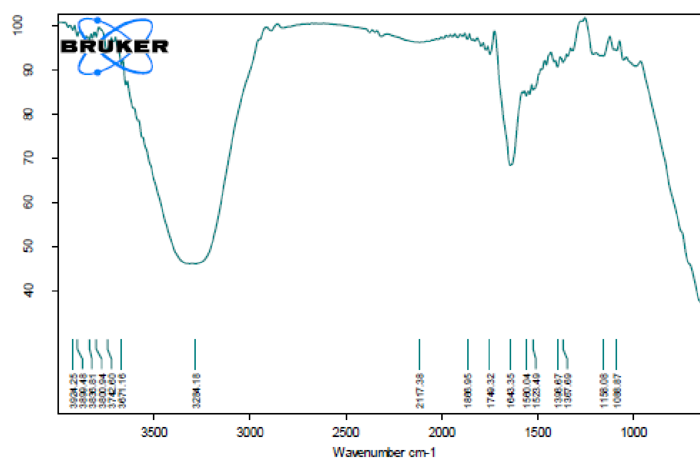

Figure 4. FTIR spectrum for Isolate 5

Table 4. FTIR Spectral Data and Functional Group Identification for Isolate 5

Wavenumber (cm^{-1})	Vibration Mode	Functional Group	Assignment
3349.14	O–H stretching	Hydroxyl	Phenolic groups
2873.11	C–H stretching	Alkane (Csp^3–H)	Aliphatic chain
1716.36	C=O stretching	Carbonyl	Conjugated ketone (C-ring)
1651.89	C=C stretching	Aromatic	Benzene ring system
1462.29	C–H bending	Alkane	Methyl/Methylene groups
1251.02	C–O stretching	Alkyl aryl ether	Ether/Phenolic linkage
901.20	C=C bending	Alkene	Substituted aromatics

3.3.3. Comparison with Quercetin

The alignment of the FTIR bands (C=O, O–H, and aromatic C=C) with the known spectral fingerprint of quercetin suggests that Isolate 5 is likely a quercetin-type flavonol or a closely related glycosidic derivative. Previous literature has consistently identified quercetin as a major bioactive in *Moringa* leaves. The chromatographic and spectroscopic data, when synthesized, suggest that the isolation process achieved a relative purity of 77.99% for this specific flavonol.

Table 5. Comparison of Isolate 5 vs. Quercetin Standard

Parameter	Isolate 5 Value	Literature/Standard (Quercetin)
UV λmax (Band II)	274.0 nm	250–275 nm
Dominant FTIR Peak	3349.14 cm^{-1} (O-H)	3400 cm^{-1} (O-H)
Chromatographic Rf	0.56	0.54
Relative Purity	77.99%	100%
Probable Class	Flavonol	Flavonol

4. Conclusion

The systematic extraction and chromatographic fractionation of *Moringa oleifera* leaves showed the presence of a diverse polyphenolic profile, with a specific emphasis on flavonol-type compounds. The use of 96% ethanol as a primary solvent facilitated the recovery of a bioactive-rich crude extract, which was effectively resolved using silica gel column chromatography. Among the isolated fractions, Isolate 5 is found to be beneficial due to its high total flavonoid content and relative purity of 77.99%. Spectroscopic

characterization through UV-Vis and FTIR confirmed the presence of characteristic structural markers, including phenolic hydroxyl groups and conjugated carbonyl systems, which strongly align with the molecular fingerprint of quercetin and its derivatives. These findings reinforce the status of *Moringa oleifera* as a potent source of natural antioxidants and provide a rigorous scientific justification for its development into therapeutic agents and functional food supplements. The high concentration of flavonoids identified in specific fractions suggests that targeted isolation can enhance the pharmacological efficacy of *Moringa*-derived products in managing oxidative stress and inflammatory disorders.

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