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Review Article

TLC Fingerprinting of *Moringa oleifera* Powder with Quercetin as the Reference Marker: A Review

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ABSTRACT

Moringa oleifera Lam. leaf powder is sold widely as a nutraceutical, but its quality depends on origin, processing and possible adulteration. Thin-layer chromatography (TLC) and high-performance TLC (HPTLC) offer an inexpensive way to check such powders, and the flavonol quercetin is an attractive reference marker because it is a pure, well-characterised compound related to the main flavonoids of the leaf. This review examines published work on the flavonoid chemistry of the leaf, the suitability of quercetin as a marker and the TLC and HPTLC systems reported for *Moringa* extracts. Leaf quercetin and kaempferol occur chiefly as glycosides, with reported contents of 0.46-16.64 mg/g and 0.16-3.92 mg/g dry weight respectively. Silica gel 60 F254 plates developed with toluene, ethyl acetate, methanol and formic acid mixtures can resolve quercetin from rutin, gallic acid and chlorogenic acid. However, compiled retardation factor (R_f) data show that values cannot be carried from one system to another: rutin moved from 0.09 to 0.51 between reported systems, and the same value of 0.72 was assigned to quercetin in one report and to chlorogenic acid in another. Reported quercetin content also varied from 993.5 µg/g in one extract to a level too low to be a significant constituent in another. Comparison with quercetin is therefore valid only on the same plate, supported by a co-spotted lane and preferably a hydrolysed extract. Few of the reports compiled here combined a full band profile with a quercetin standard on one plate. Agreement on extraction, mobile phase and derivatisation, followed by validation and confirmation of band identity by HPLC or LC-MS, is needed before fingerprints can be compared between laboratories.

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INTRODUCTION

The name on a label and the look of a powder do not establish that a herbal raw material is genuine or of acceptable quality, so a chemical identity check is needed. A chromatographic fingerprint serves this purpose. It is the pattern of bands or peaks given by an extract under fixed conditions, and it can be compared across batches and with reference standards. Such fingerprints are used in the quality control and stability testing of herbal medicinal products[1].

Moringa oleifera Lam., known as the drumstick or horseradish tree, is native to South Asia and is valued as both food and medicine[2]. Its leaf powder is the most heavily exploited form, and the commercial supply is uneven. When 29 products were examined by HPTLC, HPLC, UV-Vis spectroscopy and principal component analysis, only three gave patterns resembling an authentic ethanol extract[3]. This finding gives a practical reason for fingerprinting *Moringa* powder routinely.

Quercetin is a sensible marker for this work. It is well defined and available as a pure standard, and it is structurally related to the principal leaf flavonoids, which are mainly glycosides of quercetin and kaempferol[4,5]. TLC is the natural entry point, since sample preparation is simple, operating cost is low and the chromatogram can be inspected directly[6]. This review examines how far TLC and HPTLC data support such a comparison. It summarises the flavonoid chemistry of the leaf and the properties of quercetin, outlines the principle of the technique, compiles reported solvent systems and R_f values, and proposes a procedure for a comparative fingerprint study.

LITERATURE SEARCH

A narrative literature search was conducted using PubMed, PubMed Central (PMC), Scopus and Google Scholar. Search terms included combinations of "*Moringa oleifera*", "quercetin", "flavonoids", "thin-layer chromatography", "TLC", "HPTLC", "fingerprinting" and "standardisation". Publications reporting the phytochemical composition of *Moringa oleifera* or its TLC/HPTLC chromatographic characteristics were considered relevant. Information on plant material, extraction solvents, stationary phases, mobile phases, detection methods and reported R_f values was extracted and compared. Studies providing relevant chromatographic conditions or analytical results were included in the review.

FLAVONOID CHEMISTRY OF *MORINGA OLEIFERA* LEAF

M. oleifera belongs to the family Moringaceae and tolerates a wide range of tropical and subtropical conditions. Its bioactive compounds fall mainly into flavonoids, phenolic acids, glucosinolates, terpenes, alkaloids and sterols[2]. Composition changes with plant part and growing conditions, so any study of a powder must state which part was used.

Outside the roots and seeds, kaempferol and quercetin are present mostly in glycosidic forms such as glucosides, rutinosides and malonyl glucosides. Leaf levels have been given as 0.46-16.64 mg/g dry weight for quercetin and 0.16-3.92 mg/g for kaempferol[5]. Methanolic leaf extract contains chlorogenic acid, rutin, quercetin glucoside and kaempferol rhamnoglucoside[4], and a 2023 survey listed 46 flavonoids identified in leaves and seeds by chromatographic and mass spectrometric methods[7]. Paper chromatography has also shown flavonoids in leaf and flower[8]. These constituents are linked with the antioxidant, anti-inflammatory, anticancer and antihyperglycaemic activities ascribed to the genus[9]. For the analyst, the important point is that a leaf extract holds a family of quercetin derivatives of differing polarity and will not give one single quercetin band.

QUERCETIN AS THE REFERENCE MARKER

Quercetin ($C_{15}H_{10}O_7$) is a flavonol bearing hydroxyl groups at positions 3, 5, 7, 3′ and 4′[10,11]. These groups, together with the C2-C3 double bond and the C4 carbonyl, are credited with its antioxidant action[10]. It is widely distributed in plant foods[10], scavenges reactive oxygen species and influences glutathione levels[12,13]. Attachment of a sugar makes the molecule more polar, and this is precisely the change that a silica plate detects.

Three features favour quercetin as a TLC reference: it is a single pure compound of known structure, it belongs to the same chemical series as the main leaf flavonoids, and flavonol zones are readily seen under

UV light after derivatisation. Its limitation is equally clear. It represents only one member of a complex flavonoid profile, so a match indicates a quercetin-type compound and does not authenticate the whole powder.

PRINCIPLE OF TLC AND HPTLC FINGERPRINTING

In TLC the sample is applied to a thin adsorbent layer, usually silica gel, and the mobile phase rises through the layer by capillary action. Solutes separate according to their affinity for the polar stationary phase and the less polar solvent. Flavonoid glycosides are held strongly and move a short distance, whereas the aglycone travels further. Each zone is described by its retardation factor:

R_f = distance travelled by the compound / distance travelled by the solvent front

R_f lies between 0 and 1 and is reproducible only when the plate, solvent composition, chamber saturation, temperature and development distance are held constant. HPTLC is the instrumental form of the same method, using finer plates, automated band application and densitometric scanning. *Moringa* studies have used CAMAG systems with a Linomat applicator, TLC Scanner 3 and winCATS software[14], while a video-documentation system has been used for fingerprint capture at 254 nm, 366 nm and in white light[6]. A fingerprint is judged on the number of bands, their R_f values, their colour before and after derivatisation, and their relative intensity.

REPORTED CHROMATOGRAPHIC SYSTEMS FOR *MORINGA OLEIFERA*

Published work on *Moringa*18 Nov 2026

Table 1: Reported TLC and HPTLC Systems for *Moringa oleifera* Extracts

Reference	Sample	Stationary and mobile phase	Detection	Main result
Dutt, 2021[15]	Leaf bioactives	Silica gel 60 F254; toluene:ethyl acetate:methanol:formic acid (7:6:2:1)	254 nm	R_f 0.72 quercetin, 0.09 rutin, 0.55 gallic acid, 0.15 chlorogenic acid; 100-600 ng/band; RSD <2%
Thomas et al., 2020[14]	Ethanolic and aqueous leaf extracts	Silica gel 60 F254; toluene:ethyl acetate:methanol:formic acid (4.9:4.1:2:0.5)	300 nm	R_f 0.80 quercetin, 0.64 gallic acid, 0.22 rutin; quercetin 993.5 and 832 µg/g; RSD <2%
Alam et al., 2020[16]	Leaf, stem, flower and root	Silica gel 60 F254; ethyl acetate:acetone:water:formic acid (6:3:2:2)	254 nm	R_f 0.51 rutin, 0.72 chlorogenic acid, 0.83 gallic acid; 100-700 ng/spot
Vongsak et al., 2013[17]	70% ethanolic leaf extracts from 12 locations	Silica gel 60 GF254; ethyl acetate:formic acid:acetic acid:water	340 nm	Mean isoquercetin 0.0427%, astragalin 0.0534%, crypto-chlorogenic acid 0.0473% dry weight; RSD <5%
Bassey, 2024[3]	Ethanol and dichloromethane extracts; 29 products	Silica gel 60 F254; dichloromethane:ethyl acetate:methanol:formic acid (90:10:20:0.5)	366 nm after PEG	Quercetin and kaempferol glycosides at R_f 0.18 and 0.28 in ethanol extract, absent from dichloromethane extract
Jayani et al., 2025[6]	70% ethanol extract of 500-mesh powder	Silica gel 60 F254; toluene:ethyl acetate:methanol:formic acid (7:2:1:0.2)	366 nm	Eight bands, R_f 0.254-0.849; stability and precision met; no reference standard used
Elangovan et al., 2015[18]	Petroleum ether extract of leaf powder	HPTLC	White light	Eight components, R_f 0.24-0.99
Perera et al., 2017[19]	Methanol and dichloromethane (1:1) extract	TLC; hexane:dichloromethane:methanol (1:3.75:0.25)	UV; vanillin sulphate	Five spots under UV and ten after spraying
Uno et al., 2026[20]	Ethanolic leaf extract	Silica gel TLC with DPPH spray	UV; DPPH	Antioxidant zones located; identity of compounds not confirmed

Reference	Sample	Stationary and mobile phase	Detection	Main result
Mohsin et al., 2023[21]	Macerated and sonicated leaf extracts	TLC; toluene:butanol:methanol (90:5:5)	-	No notable profile difference between methods; quercetin not a significant constituent

CRITICAL COMPARISON OF THE REPORTED DATA

R_f values do not transfer between systems

Rutin illustrates the problem well. It ran at 0.09 in one system[15], 0.22 in a second[14] and 0.51 in a third[16]. The figure 0.72 was assigned to quercetin in one method[15] and to chlorogenic acid in another[16]. Even two closely related toluene-based mixtures placed quercetin at 0.72 and 0.80[14,15]. An R_f value taken from the literature therefore identifies nothing by itself. Within any one system the order is informative, and in the first two systems quercetin ran well above rutin, as expected from polarity. Within a single report, the extract peak may also sit slightly away from the standard: Thomas et al. gave 0.80 ± 0.03 for quercetin, while their densitogram legends list 0.76 for the extract[14]. This supports running the standard on the same plate with a co-spotted lane.

Glycoside and aglycone must be read separately

Because leaf quercetin is largely glycosidic, an extract may show a band at the aglycone position, a lower band from glycosides, or both. The low value of 0.18 reported for a quercetin glycoside[3] agrees with this behaviour in general terms, although it came from a different solvent system and cannot be set directly beside the aglycone values in Table 1. Each of the three outcomes is informative, and a hydrolysed extract spotted next to the untreated one can distinguish them, since a band appearing at the quercetin position only after hydrolysis points to quercetin glycosides.

Quercetin content and extraction

Reported quercetin ranged from 993.5 µg/g in an ethanolic extract[14] to a level too low for it to be regarded as a significant constituent in another sample[21]. A faint or absent band should therefore be reported as a result and should not be read as a failure of the method. Extraction solvent had a clear effect: rutin was 701 µg/g in the ethanolic extract and 232.2 µg/g in the aqueous extract, while gallic acid was 150 and 591.1 µg/g respectively[14], and the quercetin and kaempferol glycosides were found in ethanol but not in dichloromethane extract[3]. By contrast, maceration and ultrasonic extraction gave no notable difference in TLC or HPLC profile in one comparison, although maceration gave the larger yield[21]. On this limited evidence, the choice of solvent appears to matter more than the extraction technique.

The remaining gap

The compiled reports tend to fall into two groups. Quantitative methods report only their target peaks[14-17], whereas fingerprint studies report whole profiles but may use no standard[6,18]. The commercial product study came closest to combining the two, although it used glycoside standards[3]. A complete band profile of *Moringa* powder developed beside a quercetin standard, with a co-spot and a hydrolysed extract, would address this gap directly.

SUGGESTED PROCEDURE FOR A COMPARATIVE FINGERPRINT

Table 2 combines reported conditions with common practice for flavonoid TLC. It is offered as a starting point that requires optimisation and validation in the laboratory concerned, and it does not represent verified results.

Table 2: Suggested Conditions for Comparing *Moringa oleifera* Powder with Quercetin

Step	Condition	Comment
Sample	Authenticated leaf powder, fine mesh	500-mesh powder has been used[6]; record authentication and batch
Extraction	Maceration or sonication in ethanol or methanol; filter	Keep solvent constant, as it alters the profile[3,14]

Step	Condition	Comment
Standard	Quercetin in methanol, about 0.5-1 mg/mL	Comparable strengths have been used[3,14]
Stationary phase	Silica gel 60 F254 plate	Used in most systems in Table 1
Application	Sample and standard as bands, 5-10 µL, on one plate	Include a co-spot lane of sample and standard
Mobile phase	Toluene:ethyl acetate:methanol:formic acid (7:6:2:1)	Resolves quercetin, rutin, gallic and chlorogenic acids[15]; alternative (4.9:4.1:2:0.5)[14]
Development	Saturated twin-trough chamber, fixed distance	Keep temperature and saturation time constant
Detection	UV 254 nm and 366 nm, then natural product-PEG reagent and 366 nm	Densitometry at 254 or 300 nm has been used[14,15]
Evaluation	R_f of every band; colour and intensity against quercetin	Add a hydrolysed extract lane to confirm glycosides

A sample may be considered consistent with the marker when it shows the same R_f within the variation of the validated method, the same colour at 366 nm after derivatisation and a single merged band in the co-spot lane.

VALIDATION

A fingerprint supports quality control only if it is validated. Planar fingerprinting has been addressed through analyte stability, selectivity, robustness and reproducibility[1], and a current reference for analytical procedures is ICH Q2 (R2)[22]. *Moringa* methods have followed this pattern: one reported linearity, accuracy, precision, range and limits of detection and quantification[15], and another gave recovery of 98.1-99.4% with intra- and inter-day RSD below 2%[14]. A fingerprint-only study used acceptance limits for R_f variation of 0.02 for repeatability and 0.05 for intermediate precision[6]. These limits provide a practical benchmark for qualitative work with a quercetin standard.

ADVANTAGES AND LIMITATIONS

TLC is inexpensive, needs simple equipment and allows many samples to run beside a standard on one plate. Visual detection and derivatisation give colour information in addition to R_f, and HPTLC adds densitometry and a stored digital record. Where HPTLC and HPLC have been compared for quercetin in a plant extract, the results were close (0.33% and 0.37%)[23]. The limitations are that R_f shifts with solvent, saturation, temperature and plate batch, resolution is below that of HPLC, and co-eluting glycosides can lie under one band. A quercetin match is only a marker match, so band identity should be confirmed by HPLC or LC-MS where possible[20].

CONCLUSION

Quercetin is a practical reference marker for the TLC fingerprint of *Moringa oleifera* powder, provided the comparison takes account of the glycosidic form in which it mainly occurs. Silica gel 60 F254 methods can separate quercetin from rutin, gallic acid and chlorogenic acid, but compiled R_f data show that values from different systems cannot be pooled. Comparison must be made on one plate, with a co-spot and preferably a hydrolysed extract. Future work should fix one extraction method and one mobile phase, report R_f, colour and intensity for every band, validate the method against current ICH criteria, test several commercial batches, confirm band identity by HPLC or LC-MS, and extend the profile to kaempferol glycosides, rutin and chlorogenic acid so that identity does not depend on one marker.

LIST OF ABBREVIATIONS

DPPH: 2,2-diphenyl-1-picrylhydrazyl; HPLC: high-performance liquid chromatography; HPTLC: high-performance thin-layer chromatography; ICH: International Council for Harmonisation; LC-MS: liquid chromatography-mass spectrometry; PEG: polyethylene glycol; R_f: retardation factor; RSD: relative standard deviation; TLC: thin-layer chromatography; UV: ultraviolet.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DECLARATION ON USE OF AI TOOLS

ChatGPT (OpenAI) and Claude (Anthropic) were used to assist with language editing, manuscript organisation, editorial refinement, rewriting of text and checking of references. The authors reviewed and verified the content and take full responsibility for the final manuscript.

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