# PHYTOCHEMICAL SCREENING AND QUANTITATIVE ESTIMATION OF QUERCETIN AND GALLIC ACID IN ETHANOLIC EXTRACT OF *Moringa oleifera* LEAVES BY HPTLC


## ABSTRACT

*Moringa oleifera* (family Moringaceae), commonly known as the "Miracle Tree," is widely recognized for its rich nutritional and therapeutic properties. The present study investigated the phytochemical composition and quantified major antioxidant compounds, quercetin and gallic acid, in ethanolic leaf extract of *M. oleifera* using High-Performance Thin Layer Chromatography (HPTLC). Preliminary phytochemical screening confirmed the presence of carbohydrates, phenols, tannins, triterpenoids, flavonoids, and alkaloids. Quantitative estimation by HPTLC revealed quercetin and gallic acid contents of 860 µg/g (0.08%) and 273 µg/g (0.03%), respectively. The results validate the antioxidant richness of *M. oleifera* and support its potential application in pharmaceutical and nutraceutical formulations.


**Keywords:** *Moringa oleifera*, HPTLC, phytochemicals, quercetin, gallic acid, antioxidant potential

## INTRODUCTION

*Moringa oleifera*, a member of the Moringaceae family, is a tropical and subtropical plant recognized for its diverse nutritional and medicinal applications. Commonly known as the "Miracle Tree" or "Drumstick Tree," it is a highly valued species rich in essential nutrients such as β-carotene, amino acids, vitamins, proteins, polyphenols, flavonoids and natural antioxidants. Extensive research has documented its anti-inflammatory, anticancer, hepatoprotective, neuroprotective and anti-aging effects. Due to its broad therapeutic potential and nutritional value, *Moringa oleifera* has become increasingly important in both

the food and pharmaceutical sectors, as well as in traditional medicine, making it an economically viable crop for local cultivation and utilization (Azeem *et al.,* 2023).

The plant is a natural reservoir of antioxidants, including vitamin C, tocopherols, flavonoids and various phenolic compounds, which contribute to its hepatoprotective, antimicrobial, antidiabetic, anticarcinogenic and hypotensive activities (Dilawar *et al*., 2018). Different parts of the plant such as roots, gum, leaves, bark, flowers, fruits, seeds and seed oil exhibit diverse pharmacological properties and have been traditionally used to manage menstrual disorders, enhance fertility and support the cardiovascular health (Nwamarah *et al.,* 2015).

Owing to its richness in antioxidants, flavonoids and essential micronutrients, *M. oleifera* shows great promise in mitigating oxidative stress and inflammatory conditions. The therapeutic efficacy of the plant is primarily attributed to its high content of phenolic and flavonoid compounds, which underpin its strong antioxidant activity. The present investigation, therefore, aimed to conduct a preliminary phytochemical screening and quantitative estimation of quercetin and gallic acid, two key antioxidant biomarkers, using High-Performance Thin Layer Chromatography (HPTLC) to substantiate the phytochemical and antioxidant potential of the ethanolic leaf extract.

## METHODOLOGY

**Preliminary Phytochemical analysis** – Ethanolic extract of *Moringa oleifera* leaves was screened for presence of phytoconstituents like alkaloids, tannins, glycosides, flavonoids, saponins etc. using standard procedure (Jeevalatha *et al*., 2022).

### Test for Phenol (Ferric chloride test)

To 1 ml of the extract, 3 ml of distilled water was added followed by few drops of 10 per cent aqueous ferric chloride solution. Formation of green colour indicates the presence of phenols.

### Test for Flavonoids (Shinoda test)

To 2 ml of the extract, 1 ml of 1 per cent ammonia solution was added. Appearance of yellow colour indicates the presence of flavonoids.

### Test for Tannins (Ferric chloride test)

To 1 ml of the extract,1 ml of 0.008 M potassium ferricyanide was added and then 1 ml of 0.02 M ferric chloride containing 0.1 N HCl was added. Appearance of blue–black colour indicates the presence of tannins.

**Test for Saponins (Foam test)**

2 ml of crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam is taken as an indication for the presence of saponins.

**Test for Triterpenoids (Salkowski test)**

Plant extract was dissolved in chloroform. $H_2SO_4$ (few drops) was added after filteration and mixed thoroughly. Golden color appeared to indicate the presence of triterpenoids

**Test for Carbohydrates (Molicsh tset)**

The aqueous solution and 70% ethanol MoLE was treated with Molicsh's reagent. After dissolving in 5 ml distilled water, a purple ring appeared. Now mix few drops of alcoholic α naphthol solution in a test tube to show the presence of carbohydrates.

**Test for Phytosterols (Liebermann-Burchard test)**

Add Chloroform in plant extract and filter. A few drops of acetic anhydride was mixed, boiled and cooled rapidly. Finally, $H_2SO_4$ was mixed till brownish ring appeared.

**Test for Alkaloids (Mayer's test)**

Approximately, 1 ml of crude extract was mixed with 2 ml of wagner's reagent. Appearance of reddish brown precipitate indicates the presence of alkaloids.

**Quantification of Quercetin and total phenols -**

**Standard Preparation:** Standard solutions of quercetin and gallic acid (total phenolic content) were prepared at a concentration of 50 µg/ml by accurately weighing 5 mg of each compound, dissolving them in methanol and making up the volume to 100 ml with methanol. The resulting solutions were filtered through a 0.45 µm membrane filter to ensure clarity. Serial dilutions of the standard solution were then prepared to obtain concentrations of 100, 200, 300, 400, 500 and 600 ng for calibration purposes.

**Sample Preparation:** For the test sample, 10 mg of the ethanolic extract of *Moringa oleifera* leaves was accurately weighed, dissolved in 10 ml of ethanol and filtered through a 0.45 µm microfilter prior to chromatographic analysis.

**Chromatographic Conditions:** HPTLC analysis was performed using a CAMAG Linomat 5 system and WinCATS software.

- **Stationary phase:** Precoated silica gel 60 F254 plates
- **Mobile phase:** Toluene : Ethyl acetate : Methanol : Formic acid (4.9 : 4.1 : 2 : 0.5)
- **Chamber saturation:** 45 min
- **Solvent front distance:** 80 mm
- **Detection wavelength:** 375 nm (quercetin) and 254 nm (gallic acid)

The method exhibited linearity in the range of **2–10 µg/spot for quercetin** and **2–6 µg/spot for gallic acid**.

**Procedure:** The quantification of quercetin and total phenolic content in the ethanolic extract of *Moringa oleifera* leaves was carried out following the method described by Sulastri *et al.* (2018). HPTLC was employed as an effective analytical technique for phytochemical evaluation. The analysis was conducted using a CAMAG Linomat 5 applicator integrated with WinCATS software to ensure precise sample application and data processing. The extracts were spotted as narrow bands on precoated silica gel 60 F254 plates and developed in a solvent system comprising toluene: ethyl acetate: methanol: formic acid (4.9:4.1:2:0.5). Densitometric scanning was performed using a CAMAG TLC scanner at 362 nm to record absorbance values. Quantification was based on the measurement of spot intensity, with concentrations determined through a linear regression equation derived from standard calibration curves. This HPTLC protocol enabled accurate and reproducible quantification of quercetin and total phenols in the ethanolic extract.

## RESULTS AND DISCUSSION

A preliminary phytochemical screening of the ethanolic leaf extract revealed the presence of key bioactive constituents, particularly the flavonoid quercetin and the phenolic compound gallic acid. The presence and concentration of these compounds were further verified using High-Performance Thin Layer Chromatography (HPTLC), confirming the extract's rich phytochemical composition.

**Preparation of Ethanolic Extract** - Cold maceration of 100 gram of dried *Moringa oleifera* leaves using ethanol resulted in a yield of 10.45 gram of extract, corresponding to an extractability of 10.45 percent, as presented in Table 01.

**Table 01: Extractability of the ethanolic extract of *Moringa oleifera* leaves**

| Plant part | Solvent for extraction | Method of extraction | Dry weight of plant material (g) before extraction | Dry weight of extract (g) | Per cent extractability |
|---|---|---|---|---|---|
| *Moringa oleifera* leaves | Ethanol | Cold - maceration | 100 g | 10.45 g | 10.45 |

**Preliminary phytochemical analysis**

The ethanolic extract of *Moringa oleifera* leaves was analyzed for the presence of various phytoconstituents. Preliminary phytochemical screening confirmed that the extract contained carbohydrates, phenols, tannins and triterpenoids in varying concentrations, with phenolic compounds, flavonoids and alkaloids being the most abundant bioactive components, as shown in Table 02. The present findings are consistent with those of K*halid et al.* (2024), who also reported the presence of phenols, flavonoids, tannins, proteins, saponins, triterpenoids, reducing sugars, carbohydrates and alkaloids in ethanolic extracts of *M. oleifera* leaves prepared by the cold maceration method. Similarly, Shahriar *et al*. (2012) identified phenols, flavonoids, tannins, alkaloids, glycosides, carbohydrates, amino acids and triterpenoids in ethanolic extracts obtained using the soxhlet extraction technique.

**Table 02: Phytochemical analysis of the ethanolic extract of *Moringa oleifera* leaves**

| S.No. | Phytochemical | Tests | Result |
|---|---|---|---|
| 1 | Phenols | Ferric chloride test | + |
| 2 | Flavonoids | Shinoda test | + |

| | | | |
|---|---|---|---|
| 3 | Tannins | Ferric chloride test | + |
| 4 | Saponins | Foam test | - |
| 5 | Triterpenoids | Salkowaski test | + |
| 6 | Phytosterols | Libermann - Buchard test | - |
| 7 | Alkaloids | Mayer's test | + |
| 8 | Carbohydrates | Molicsh test | + |

The ethanolic extraction process produced a 20% w/w yield from the dried *Moringa oleifera* leaves, aligning with previously reported values for ethanol-based extractions. Phytochemical evaluation revealed the presence of several bioactive metabolites, with flavonoids and phenolic compounds being predominant, both recognized for their potent antioxidant and hepatoprotective properties**.** These findings emphasize the phytochemical richness of *M. oleifera* leaves and reinforce their therapeutic and antioxidant potential. The identification of these compounds substantiates the plant's pharmaceutical and nutraceutical value**,** providing a scientific foundation for its traditional medicinal applications**.**

**Quantification of quercetin and total phenol (gallic acid) by HPTLC**

The reported HPTLC (High-Performance Thin Layer Chromatography) method provides a reliable and efficient approach for the simultaneous determination of gallic acid and quercetin in *Moringa oleifera* leaves, utilizing UV detection in combination with mass spectrometry. Phytochemical analysis confirmed the presence of the flavonoid quercetin and the phenolic compound gallic acid in the ethanolic leaf extract. Chromatograms of both the standards and the sample extracts displayed well-resolved, distinct peaks, with gallic acid and quercetin exhibiting symmetrical and reproducible profiles. The simultaneous separation and quantification of these compounds were successfully performed using HPTLC precoated silica gel 60 F254 aluminum plates.

The HPTLC method for the quantitative estimation of quercetin and gallic acid was optimized using a mobile phase composed of toluene: ethyl acetate: methanol: formic acid (4.9:4.1:2:0.5)**.** This solvent system enabled satisfactory separation of quercetin and gallic

acid from the extract matrix, with mean retardation factor (Rf) values of 0.48 and 0.42**,** respectively. The method demonstrated linearity within the concentration ranges of 2–10 µg/spot for quercetin and 2–6 µg/spot for gallic acid, based on their peak areas. The chromatography chamber was saturated for 45 minutes and the solvent front was allowed to travel 80 mm**.** Under these conditions, well-defined and compact spots of quercetin and gallic acid were obtained, showing optimal migration. The HPTLC chromatograms were recorded at 375 nm for quercetin and 254 nm for gallic acid and the spectra of the sample spots were compared with the corresponding standards.

The identity of quercetin and gallic acid in the ethanolic extract of *Moringa oleifera* was confirmed by overlaying the UV absorption spectra of the sample bands with those of the respective standard compounds. The purity of each compound was further verified by comparing the spectra at the start, middle and end positions of the bands. Quantitative analysis revealed that the extract contained 860 µg/g of quercetin and 273 µg/g of gallic acid, corresponding to concentrations of 0.08% and 0.03%, respectively (Table 03).

**Table 03: Concentration of Quercetin and Gallic acid in ethanolic extract of *Moringa oleifera* leaves**

| **Amount of Gallic acid (µg/gm)** | **Concentration of Gallic acid (per cent)** | **Amount of Quercetin (µg/gm)** | **Concentration of Quercetin (per cent)** |
|---|---|---|---|
| 273 | 0.03 | 860 | 0.08 |

Similarly, Niranjan *et al.* (2017) also quantified the amount of gallic acid and quercetin in ethanolic extract of *Moringa oleifera* leaves by HPTLC and the concentration was reported as 0.029 % gallic acid and 0.013 % quercetin alongwith various other phytochemicals in their respective plant sample.

The findings of Thomas *et al.* (2020) are also in agreement with the findings of present study where they reported that the ethanolic and aqueous extracts of *Moringa oleifera* exhibited higher content of quercetin (993 µg/g and 832 µg/g, respectively). While, aqueous extract of gallic acid (591 µg/g) exhibited a higher proportion as compared to ethanolic extract of gallic acid (150 µg/g).

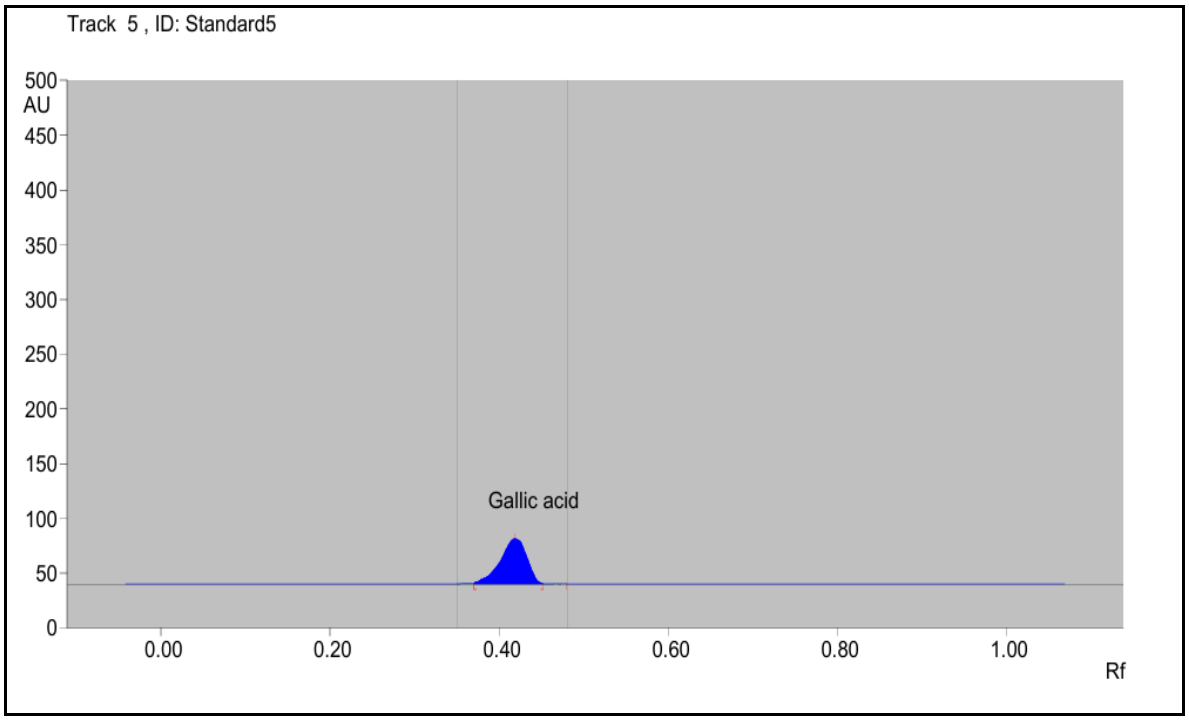


**Plate 01: HPTLC chromatogram of the standard Gallic acid (@ 254 nm)**

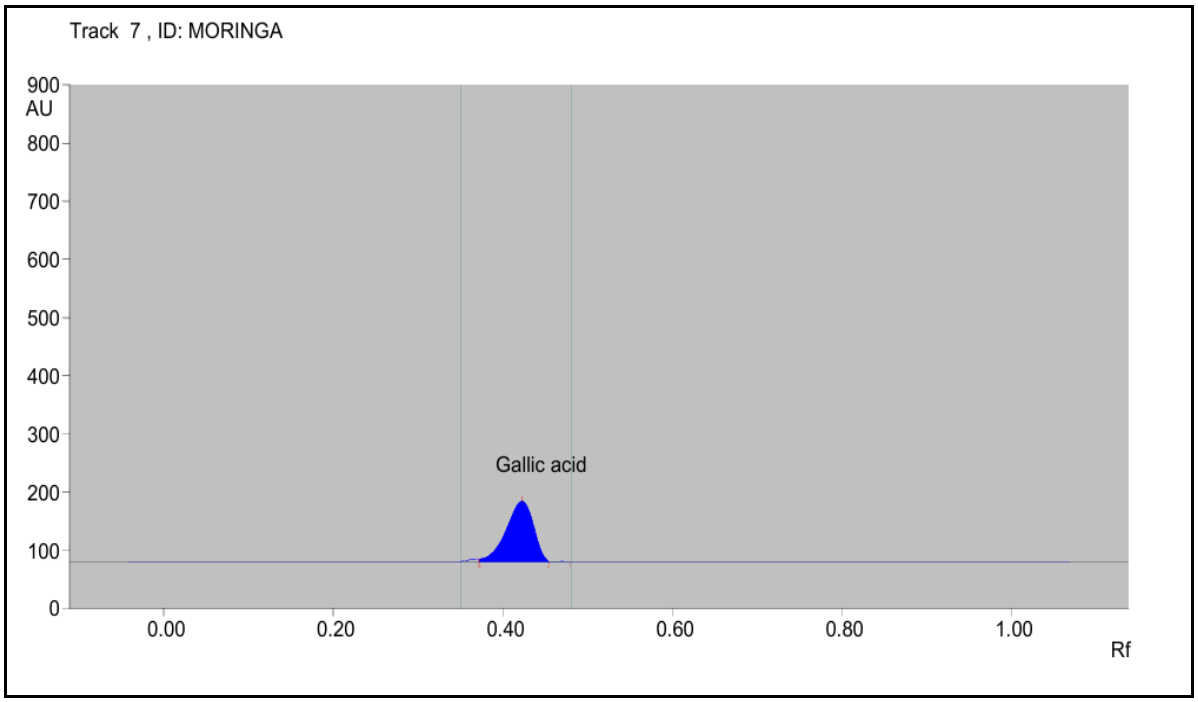


**Plate 02: HPTLC chromatogram of Gallic acid (@ 254 nm) in ethanolic extract of *Moringa oleifera* leaves**

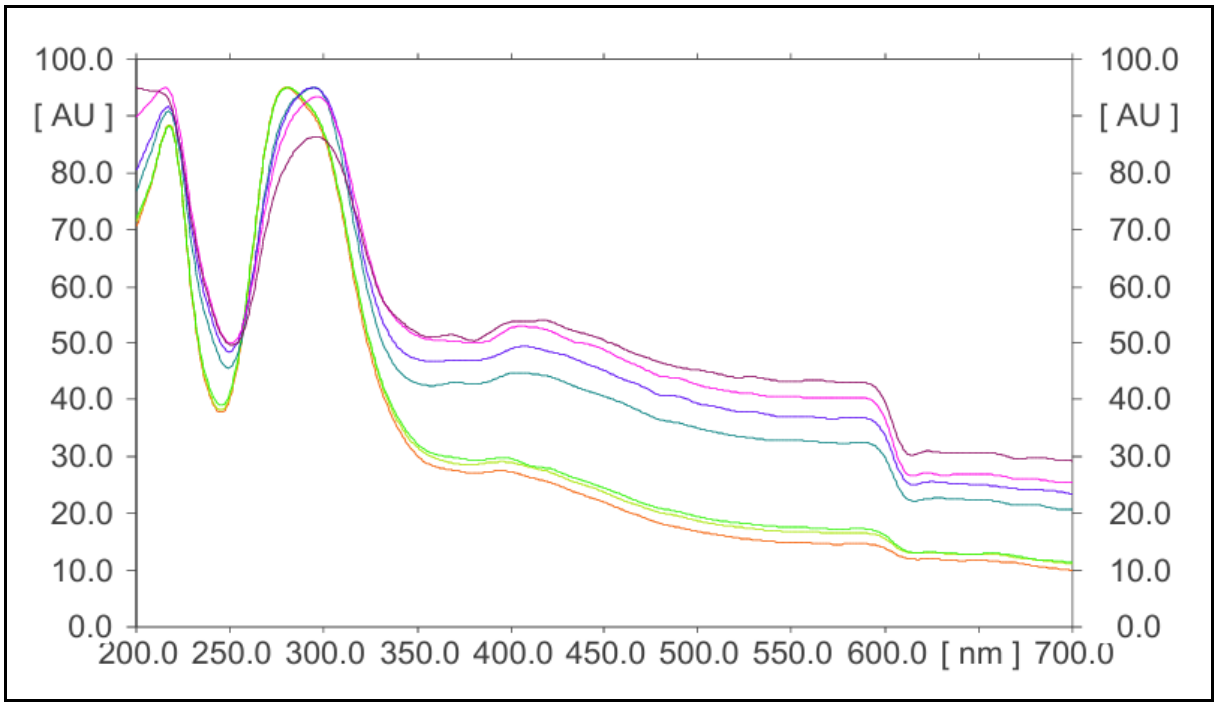


**Plate 03: Superimposable UV spectra of Gallic acid**

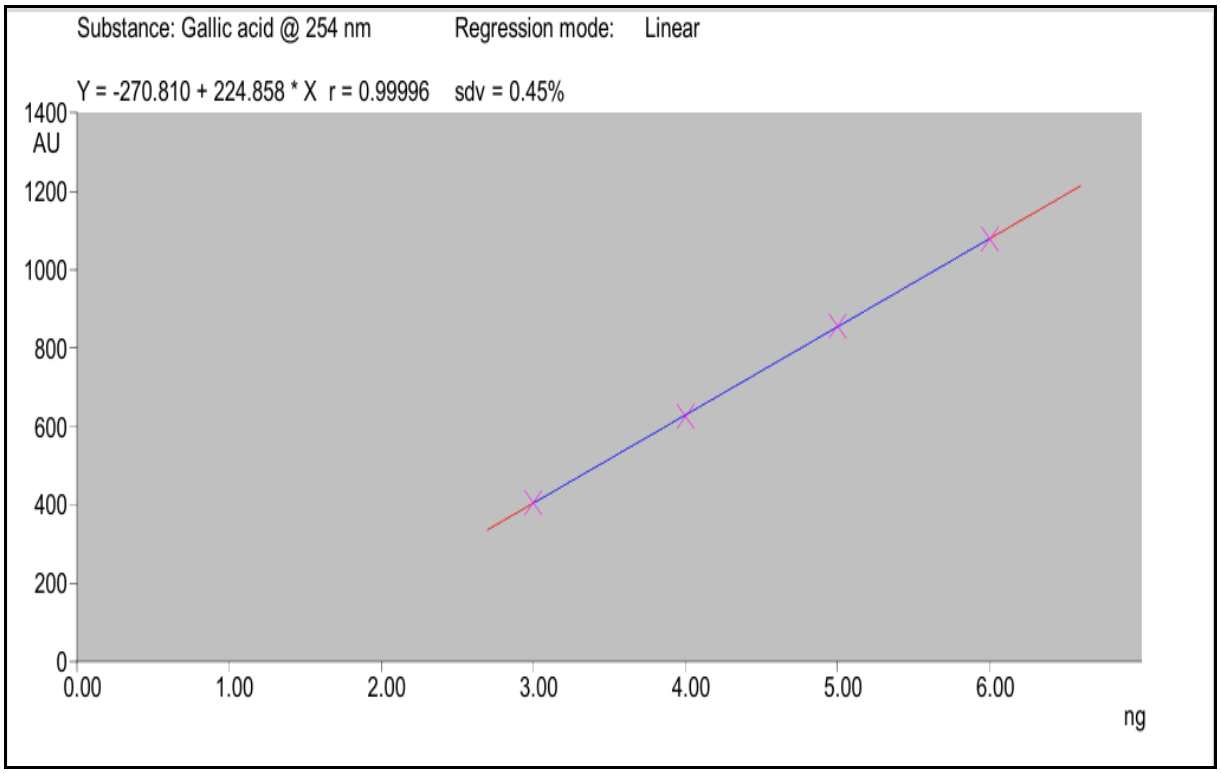


**Plate 04: Linear curve of Gallic acid**

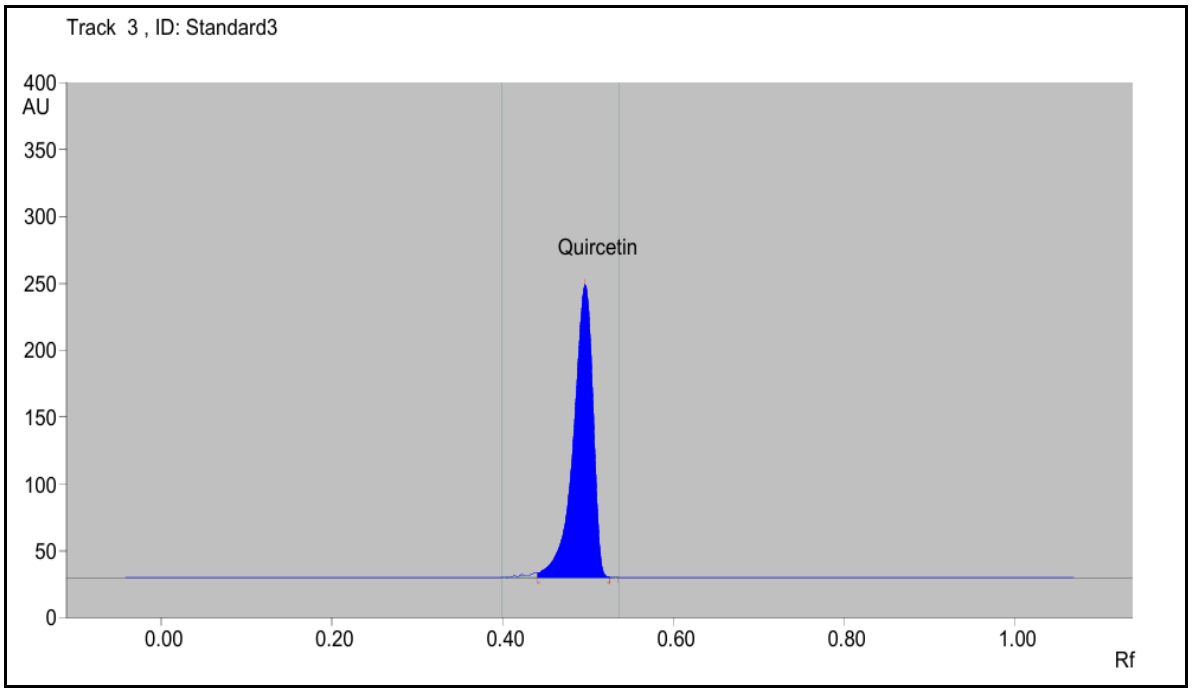


**Plate 05: HPTLC chromatogram of the standard Quercetin (@ 375 nm)**

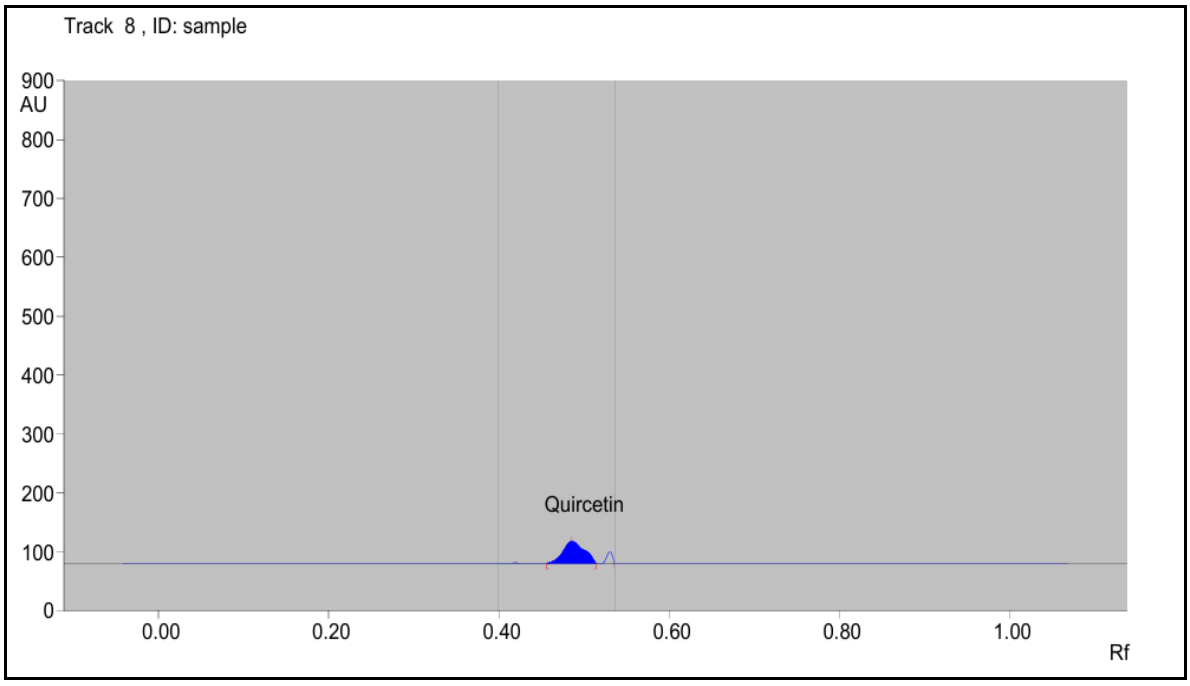


**Plate 06: HPTLC chromatogram of Quercetin (@ 375 nm) in ethanolic extract of *Moringa oleifera* leaves**

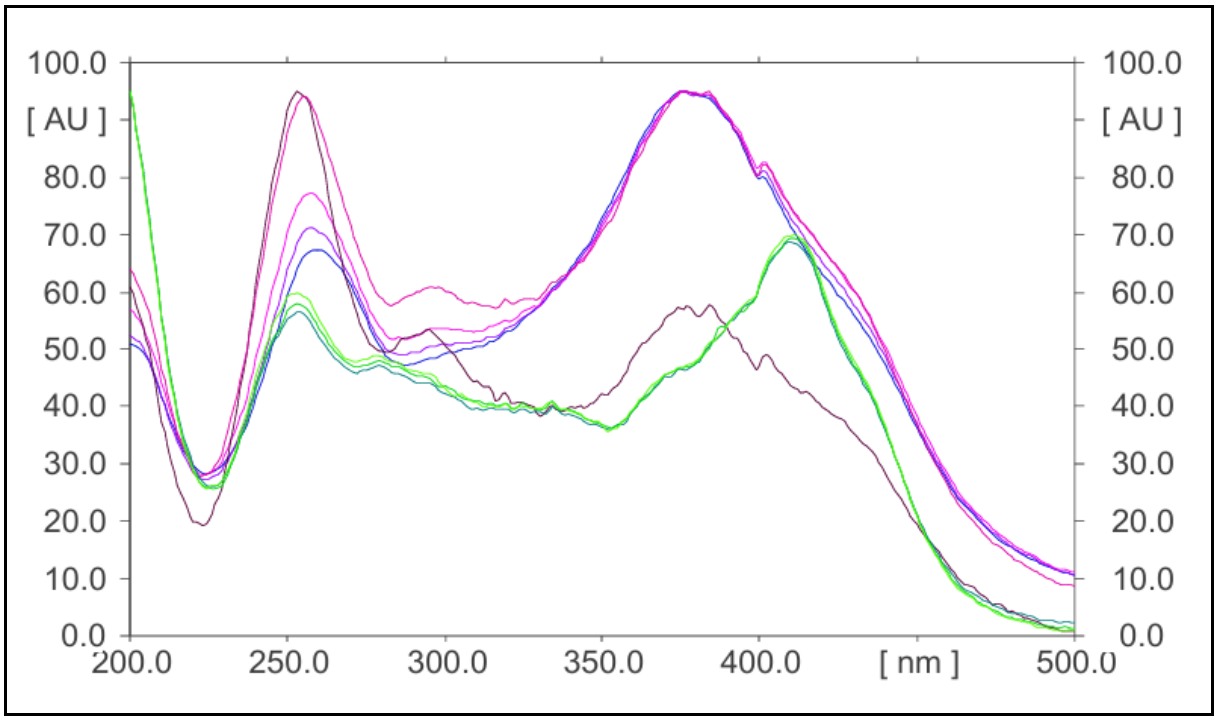


**Plate 07: Superimposable UV spectra of Quercetin**

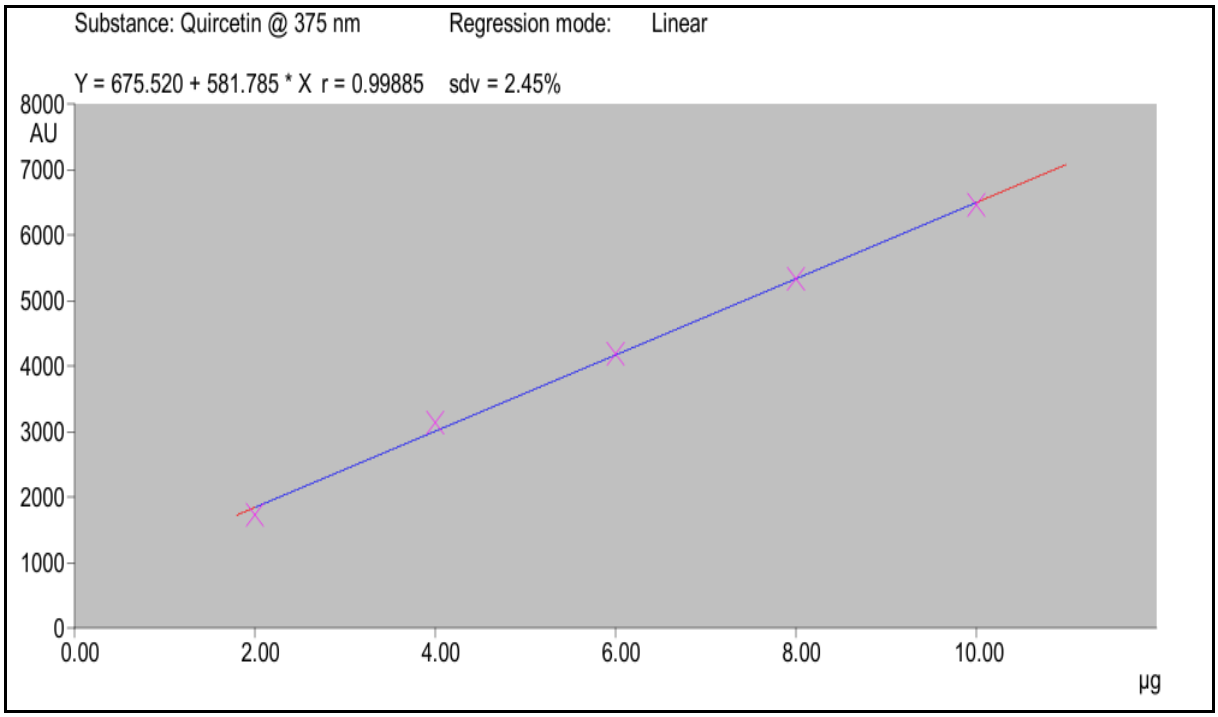


**Plate 08: Linear curve of Quercetin**

## CONCLUSION

Ethanolic extraction of *Moringa oleifera* leaves yielded an extract rich in phytochemicals, including carbohydrates, phenols, tannins, triterpenoids, flavonoids and alkaloids, with phenolic and flavonoid compounds being predominant. HPTLC analysis confirmed the presence of quercetin and gallic acid, with concentrations of 860 µg/g (0.08%) and 273 µg/g (0.03%) of the extract, respectively, validating the antioxidant potency of the preparation. These findings support the therapeutic and nutraceutical applications of *M. oleifera* and provide scientific evidence for its traditional medicinal use.

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