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RESEARCH ARTICLE

Qualitative and Quantitative Estimation of Immunomodulatory Flavonoid Quercetin from Different Parts of *Colocasia esculenta* (L.) using Validated HPTLC Method

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Abstract

Background: *Colocasia esculenta* (L.), which is commonly called taro is a significant dietary source in West Bengal, India, and holds potential health benefits due to its bioactive compounds. Quercetin, a flavonoid with immunomodulatory properties, is known to be present in taro, but its distribution and concentration in different parts of the plant remain unclear. **Aim:** This research aims to create a straightforward, efficient, and dependable technique for quantifying quercetin, an immunomodulatory flavonoid, in various sections of *C. esculenta* (L.). **Method:** A validated HPTLC technique was established using TLC silica gel plates as a stationary phase and a mobile phase tailored for the separation of quercetin. Qualitative analysis identified quercetin based on its R_f value and fluorescence characteristics compared to a standard. **Result:** This method was checked to be precise, specific, and accurate across various measurements. Recovery studies showed an average of 98.7% of quercetin is retrieved accurately. Different parts of *C. esculenta* (L.) were found to contain 0.16- 1.76 mg/100g of quercetin. **Conclusion:** This novel HPTLC method is an efficient and reliable tool for routine analysis of quercetin in various plant samples, extracts, and even commercially available products. This is one of the most efficient techniques for this particular purpose, potentially setting a precedent for quality control and standardization of herbal products containing *C. esculenta* (L.).

Major Findings: This study developed a validated HPTLC method for the qualitative and quantitative estimation of quercetin in different parts of *C. esculenta* (L.). The highest concentration of quercetin was found in the rhizomes (1.76 mg/100g), followed by the leaves (1.39 mg/100g), stems (0.44 mg/100g), and roots (0.16 mg/100g), providing new insights into the plant's potential health benefits.

Keywords: Bioactive, *C. esculenta*, Distribution, HPTLC, Immunomodulatory, Quercetin, Validated

1. Introduction

Looking back, nature has been a rich source of medicine for eons, giving rise to countless modern drugs drawn from its bounty. With 80% of the world still turning to traditional practices for health and healing, nature's potential as a pharmacy for the future remains vast[1]. Recently, there has been a growing recognition of the potential therapeutic benefits that medicinal plants could offer and indicate promising directions for future health management. The identification of new chemical structures possessing pharmaceutical activities is often serendipitous, and increasing the likelihood of such discoveries involves the systematic screening of a large number of compounds. Notably, the secondary metabolites derived from plant sources, known as plant-based drugs, have made substantial contributions to modern therapeutics[2]. *C.esculenta* (L) is a prominent example of such a plant with versatile applications. This tropical plant is predominantly cultivated for its edible corms, commonly referred to as taro[3]. Art of the *Araceae* family and the *Aroideae* subfamily, taro is a wetland

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herbaceous perennial native to tropical and equatorial areas and are mostly found in subtropical climates. Southeast Asia is a major producer of this crop, where it is known by various local names including Arbi, Eddode and Arvi[4]. The, steam, leaf, root and rhizomes of taro are also commonly utilized as a vegetable, often colloquially known as "elephant ears," reaching heights of 1-2 m during growth[4] (Figure 1).

Taro is found to posses superior nutritional value compared to other staples like potatoes, sweet potatoes, cassava, and rice. The plant's leaf juice serves as a stimulant, rubefacient, and styptic, proving beneficial in addressing internal adenitis, otalgia, hemorrhages, and buboes. Additionally, the juice from the corm has soothing properties, anodyne and laxative[5]. The leaves of *C. esculenta* (L.) are noted for their high nutrient content. Nutrients comprises both minerals and vitamins. The leaves are enriched with riboflavin, niacin, thiamin, ascorbic acid, iron, phosphorous and calcium. Fresh, edible leaves are also rich in dietary fiber, protein, essential minerals and ascorbic acid[6].

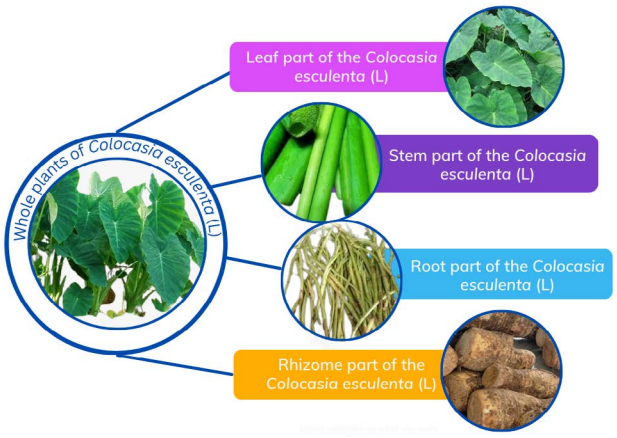


Figure 1. Different parts of *Colocasia esculenta* (L).

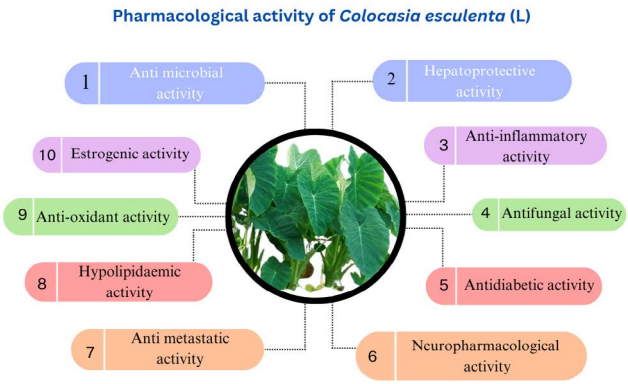


Figure 2. Different Pharmacological activity of *Colocasia esculenta* (L.).

Although the rhizome of *C. esculenta* (L.) has a comparatively low content of protein and fat, it is an important source of macronutrients like carbohydrates and minerals. Significant compounds identified in the corms up to now include cyanidin (3-rhamnoside, cyanidin 3-glucoside), and pelargonidin 3-glucoside, which is an anthocyanin. These substances enhance the plant's effectiveness in alleviating stomach inflammation, discomfort, and fever[7]. The medicinal attributes and chemical components of *C. esculenta* (L.) have been widely employed in traditional healing practices. Extracts from this plant exhibit diverse pharmacological activities, encompassing anticancer, antihyperlipidemic, antihypertensive, wound healing, antihelminthic, antimelanogenic, anti-inflammatory, hepatoprotective, antimicrobial, probiotic, hypolipidemic, anxiolytic and antidiabetic effects[8] (Figure 2).

Within the various essential bioactive compounds found in *C. esculenta* (L.), quercetin stands out as particularly significant, known for inducing intended results in users. Quercetin, classified as a bioflavonoid, has been recorded for its diverse range of health-promoting attributes. These include demonstrated anti-inflammatory, antihyperglycaemic, and antineoplastic activities, alongside other properties that contribute to enhancing mental and physical performance[9]. Moreover, research has indicated its capacity to mitigate heat sensitivity in a mouse model of diabetic nerve pain[10].

High-performance thin-layer chromatography, also called HPTLC represents a highly sophisticated, automated and efficient evolution of the conventional TLC method, boasting improved separation efficiency and lower detection limits[11]. Notably, HPTLC is faster, more straightforward, and more adaptable compared to alternative chromatographic techniques.

Consequently, in the current study, the presence of major classes of secondary metabolite, quercetin was confirmed by employing the HPTLC technique. This involved applying the technique following the extraction procedure on samples obtained from the rhizomes, roots, leaves, and stems.

The existing literature review revealed an absence of reports concerning the Thin-Layer HPTLC

chemical profile of major phytochemical, quercetin within the extracts of rhizomes, roots, leaves, and stems of *C. esculenta* (L.). The aim of the present study was to establish a sensitive, validated, and rapid HPTLC technique for the concurrent detection and measurement of quercetin in extract of rhizomes, roots, leaves, and stems *C. esculenta* (L.). This approach, introduced for the first time, demonstrates wide applicability for the routine analysis of quantification and quality control of related extracts, pharmaceutical products, and multi-herb formulations.

2. Materials and Methods

2.1 Reagents, Materials, and Standard Solutions

All chemicals and solvents used were of analytical quality and obtained from E. Merck in Darmstadt, Germany. Standard quercetin, which is a flavonoid, was obtained from Loba Chemie in Mumbai, India with a purity greater than 97%. Stock solutions containing the standard compounds at a concentration of 100 mg/ml were prepared in methanol just before they were used. TLC aluminum plates coated in advance with silica gel 60 F_{254} (10x10 cm, 0.2 mm thickness) were acquired from E. Merck Ltd (Mumbai, India).

2.2 Standard Preparation

A reference solution of quercetin (98% purity) was prepared in absolute methanol to a final concentration of 100 milligrams per milliliter. The mixture required sonication with ultrasound to achieve complete dissolution.

2.3 Sample Preparation

The four different parts of the *C. esculenta* (L.) were first dried, then coarsely ground individually. Each part was then thoroughly extracted with methanol through maceration process for seven days. The methanol was then removed completely by reducing the pressure by employing a rotary evaporator. Ultimately, each remaining sample was individually dissolved in methanol using individual 50 mL volumetric flasks[12].

2.4 HPTLC Instrumentation and Experimental Conditions

The precoated TLC (Thin-Layer Chromatography) plates of silica gel as stationary phase (E. Merck, Darmstadt, Germany) was used for this experiment. The plate dimension was $60F_{254}$, 20 cm x 10 cm, 0.2 mm thickness. These plates were first cleaned by thoroughly dipping them in methanol and air-drying them in a fume hood. Before use, the plates were activated in a hot air oven at 60°C for 5 minutes.

A semi-automated TLC sampler (Linomat V, Camag, Muttenz, Switzerland), managed by WinCATS software version 1.4.4, was utilized to deposit the sample solutions onto the plates. The application rate was maintained at a steady 80 nL s^{-1}, with a band width of 3.0 mm and a distance of 6.2 mm between bands.

Chromatography was conducted in a 20 cm x 10 cm twin trough glass chamber. The chamber was manufactured by Camag, Muttenz, Switzerland. The mobile phase of total volume 10 mL was a blend of toluene, ethyl acetate, and formic acid in the ratio of 5:4:0.2 (v/v/v). The chamber was covered to saturate with the mobile phase for 20 minutes at room temperature (25°C ± 2°C) and 60% ± 5% relative humidity. The chromatographic run extended up to 80 mm from the initial application point (10 mm)[13].

After the chromatographic run, the TLC plates were air-dried with a hairdryer set to the normal mode for 5 minutes. Densitometric analysis was carried out using a Scanner III with WinCATS software (Camag) in UV mode. Deuterium lamp was used and was adjusted to 275 nm. The slit dimensions were configured to 3.0 × 0.45 mm and the scanning speed was set at 20 mm s^{-1}. Quercetin was detected by its spots at $R_f = 0.52 \pm 0.05$.

2.5 Method Validation

2.5.1 Linearity Range

The method's consistency in linearity was validated by analyzing different standard solutions (100 mg mL^{-1}) of quercetin of five various concentration levels (100-300 μL). Linear least-squares regression was used to verify linearity and produce a calibration curve. The regression equation, including intercept, slope, and coefficient of correlation (R^2), was obtained[14].

2.5.2 Limit of Detection and Limit of Quantification

The compound was identified using R_f values and UV/Visible spectral overlays with reference substances. Diluted standards were added to HPTLC plates. The calibration curves were plotted. The LOD was defined as the minimum detectable concentration in two standards, while the LOQ was set as the lowest concentration that could be precisely measured in samples[14].

2.5.3 Accuracy

To determine the method's accuracy, pre-examined samples were spiked with standard quercetin solution and analyzed again using HPTLC. Spiking was performed at three different concentration levels, and the mean percentage recovery was determined. The procedure was conducted in triplicate[14].

2.5.4 Precession

The developed methodology was evaluated for intra assay precision by analyzing three samples of the substance at three different quercetin concentrations (100, 150, and 200 µl per band). Inter assay precision was assessed at consistent levels over three separate days in the laboratory[14].

3. Results

3.1 HPTLC Analysis

HPTLC analysis revealed that the mobile phase consisting of toluene, ethyl acetate, and formic acid in a 5:4:0.2 volume ratio successfully separated the flavonoid quercetin in the sample. This is evident in Figure 3, which depicts the thin-layer chromatography profiles of both samples observed under visible light at wavelengths of 245 nm and 366 nm.

3.2 Method Validation

3.2.1 Calibration Curve

As illustrated in Figure 4, the calibration plot demonstrates a direct proportional correlation between the response and the concentration of quercetin within the range of 100-300 µg/ml. The strength of this linear association is confirmed by a strong correlation coefficient (R^2) of 0.97. Additionally, the equation for this line is represented by a slope of 0.0000093 and an

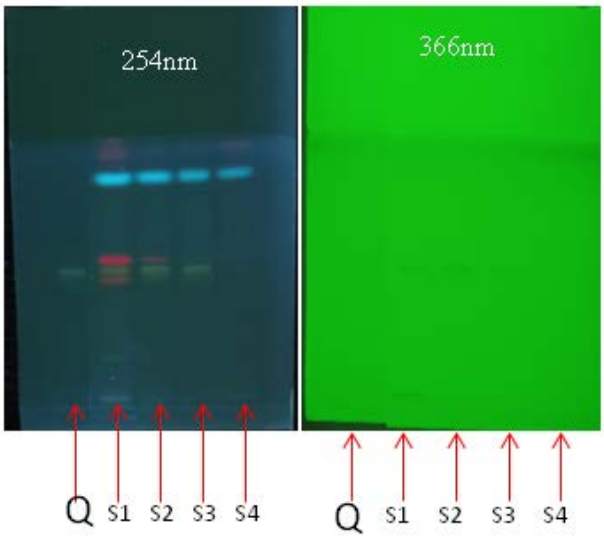


Figure 3. TLC profile in UV cabinet at 254 nm and 366 nm, Q- Quercetin, S1- Root, S2- Rhizomes, S3- Stem and S4-leaf.

Table 1. Linear Regression data for standard Quercetin

Linear Regression Parameter	Data
Linearity range (µg/spot)	100-300
Regression equation	0.00116x + 0.0000093
Correlation coefficient(R2)	0.97
Slope	0.0000093
Intercept	0.001166
SE of intercept	0.0010
SD of intercept	0.0023
LOD	0.0075
LOQ	0.0230

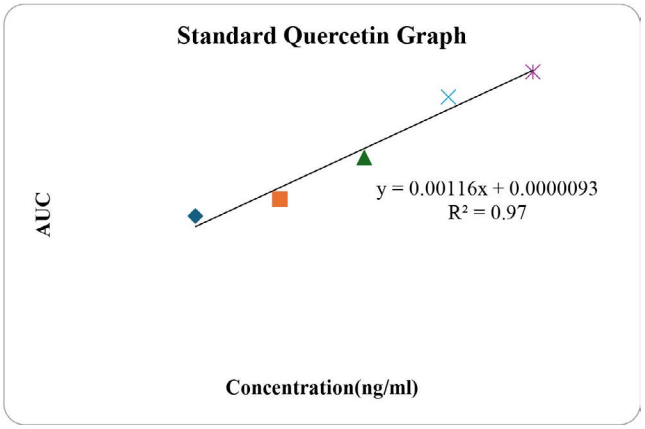


Figure 4. Standard graph of standard quercetin.

intercept of 0.001166. For further details regarding the specific data points, refer to Table 1.

3.2.2 Recovery Studies

The recovery studies presented in Table 2 yielded results within acceptable limits, achieving a value of 99.43%. This indicates that the method demonstrates good accuracy in quantifying the target analyte.

3.2.3 Precision

The results for repeatability and intermediate precision, shown as percentages of standard deviation (SD), are outlined in Table 3. The Relative Standard Deviation (RSD) varied from 0.90 to 1.35 for repeatability and from 0.80 to 1.03 for intermediate precision. These minimal values indicate a high degree of precision in the method.

3.2.4 Limit of Detection and Limit of Quantification

The proposed method demonstrated a Limit of Detection (LOD) of 0.0075 µg/spot and a Limit of Quantification (LOQ) of 0.0230 µg/spot for quercetin. These sensitive detection limits indicate the method's broad applicability for effectively detecting and quantifying quercetin across various sample types.

3.3 Method Development

To create a reliable and accurate densitometric HPTLC technique for quercetin examination, the mobile phase was carefully prepared. The combination of toluene, ethyl acetate, and formic acid in a ratio of 5:4:0.2 (v/v/v) produced a clear, symmetrical, and well-defined quercetin peak with an R_f value of 0.52 ± 0.03 (Figure 5).

Table 2. Recovery study for proposed method (n=3)

Excess drug added to analyte (%)	Concentration found(µg±SD)	% Recovery	% RSD
50	49.12±0.14	98.24	0.28
100	99.43±0.85	99.43	0.86
150	147.67±0.70	98.45	0.47

3.4 Quantitative Estimation of Quercetin form Different Parts of *C. esculenta* (L.)

The detection of quercetin in methanolic extracts from different parts of *C. esculenta* (L.) (Root, Rhizomes, Stem, and Leaf) was verified by comparing their single spot at R_f = 0.52 ± 0.03 (Figure 6) with the corresponding spot from the standard under the same chromatographic conditions. The amount of quercetin in these extracts was determined by referencing the standard's concentration and the area under the curve (AUC), as detailed in Table 4.

4. Discussion

This study established a validated HPTLC method for identifying and quantifying quercetin, a significant bioactive compound, in various parts of *C. esculenta* (L.). The mobile phase composition effectively separated quercetin, with a single peak observed at R_f = 0.55 ± 0.03. The method demonstrated good linearity, accuracy, precision, specificity, and sensitivity, making it suitable for routine analysis.

Quercetin was successfully identified and quantified in all analyzed parts of the plant, with

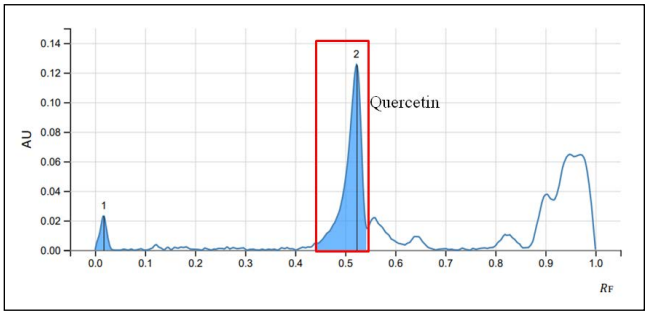


Figure 5. HPTLC chromatogram of standard quercetin.

Table 3. Precision of the proposed method (n=3)

Concentration (mg/ml)	Repeatability (Intra-day precision)			Repeatability (Intra-day precision)		
	Area±SD	Std. error	%RSD	Area±SD	Std. error	%RSD
100	0.0085±0.025	0.00014	0.9878	0.0082±0.03	0.0002	0.9249
100	0.0081±0.028	0.00017	1.3352	0.0085±0.03	0.0002	0.8848
100	0.0084±0.032	0.00019	1.3162	0.0091±0.04	0.00023	1.018

Table 4. Quantity of quercetin from different parts of *Colocasia esculenta* (L.)

Sl. No.	Parts of *Colocasia esculenta* (L.)	Quercetin Content (Mean ± SD, mg/100g dry weight)
1	Rhizomes	1.76±0.03
2	Leaf	1.39±0.06
3	Stem	0.44±0.01
4	Root	0.16±0.05

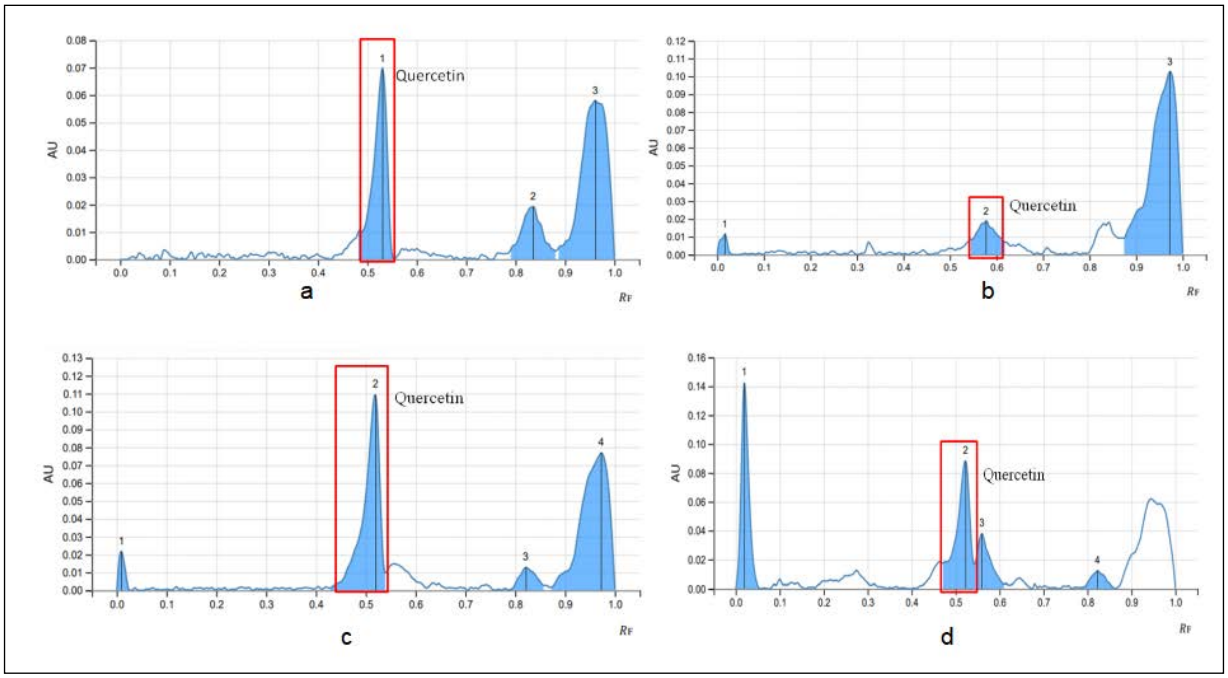


Figure 6. HPTLC chromatogram of methanolic extract of *Colocasia esculenta* (L.) different parts: **(a).** Root part, **(b).** Stem part, **(c).** Rhizome part and (d) Leaf part.

varying concentrations. The highest amount was observed in the rhizomes (1.76 mg/100g), followed by leaves (1.39 mg/100g), stems (0.44 mg/100g), and roots (0.16 mg/100g). These findings contribute to the understanding of quercetin distribution within *C. esculenta* (L.) and its potential health benefits associated with different plant parts.

All the plant parts are widely consumed by the people all over the country. The present study indicates the quantitative distribution of the important flavonoids Quercetin among different parts which will help to suggest nutrition preferences for the consumers and quantification is health benefits.

5. Conclusion

This study effectively developed a validated HPTLC method for both the qualitative and quantitative analysis of quercetin in different parts of *C. esculenta* (L.). The method demonstrated good linearity, accuracy, precision, specificity, and robustness, ensuring reliable results. Quercetin was identified and quantified in all analyzed parts, with the highest concentration found in the rhizomes (1.76 mg/100g), followed by leaf (1.39 mg/100g), stem (0.44 mg/100g), and root (0.16 mg/100g). This research contributes valuable information on the quercetin content of different parts of *C. esculenta* (L.) parts, potentially paving the way for further exploration of its potential health benefits and applications.

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7. Author Conribution

Tushar Adhikari: Conceptualization, investigation, writing - original draft. Prerona Saha: Formal analysis, supervision, writing - review and editing. All authors have read and agreed to the published version of the manuscript.

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