Qualitative and Quantitative Estimation of Gallic Acid from Colocasia esculenta Extract Using HPTLC Method

[1]Dr.Kumari Priyanka [1]Dr. Md. Sarfaraz Ahmad*

[1] Department of Botany, Jai Prakash University, Chapra (Bihar)

sushilpriyanka29@gmail.com ; mdsarfarazahmad786@gmail.com

Abstract:

Colocasia esculenta (L.), commonly known as taro, is a nutritionally and medicinally important plant containing diverse bioactive compounds. Among these, gallic acid is a key phenolic constituent known for its potent antioxidant, anti-inflammatory, and antimicrobial properties. The present study aimed to develop and validate a simple, precise, and reproducible High-Performance Thin-Layer Chromatography (HPTLC) method for the qualitative and quantitative estimation of gallic acid from the methanolic extract of *C. esculenta*. Chromatographic separation was achieved on silica gel 60 F_{254} plates using a mobile phase of toluene: ethyl acetate: formic acid: methanol (3:3:0.8:0.2 v/v/v/v), and densitometric scanning was carried out at 254 nm. A distinct peak of gallic acid was observed at Rf = 0.51 ± 0.02, confirming its presence in the extract. The calibration curve showed good linearity in the range of 100–500 ng/spot, with the regression equation $y = 1.41x - 44.333$ and correlation coefficient $R^2 = 0.998$. The gallic acid content in the methanolic extract was found to be 357 ng/spot. Validation parameters, including recovery (98.3–99.6%), %RSD (<2.0), LOD (0.007 µg/spot), and LOQ (0.023 µg/spot), confirmed the method's accuracy, precision, and sensitivity. Thus, the developed HPTLC method is reliable, cost-effective, and suitable for routine qualitative and quantitative analysis of gallic acid in *Colocasia esculenta* and related herbal formulations.

Keywords: Colocasia esculenta, Extraction, HPTLC, Qualitative and Quantitative Estimation of Gallic Acid.

Introduction

Nature has long served as a valuable source of bioactive compounds, many of which have been instrumental in the development of modern medicines. Medicinal plants continue to play a vital role in traditional and modern healthcare systems due to their therapeutic efficacy and minimal side effects. Among such plants, Colocasia esculenta (L.), commonly known as taro, occupies an important position as both a food crop and a medicinal resource. Widely cultivated in tropical and subtropical regions, C. esculenta is valued not only for its edible corms but also for its leaves, stems, and roots, all of which are rich in essential nutrients and phytochemicals (Arulmozhi *et al.* 2007). 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 (Hwang *et al*. 2014).

Colocasia esculenta (*C. esculenta*) is a widely cultivated plant for consumption and traditional medicine. Recent evidences showed lack studies about the stem of *C. esculenta* as medicinal agent. Besides, ice cream is a food, which contains milk that play beneficial role as antioxidant (Asaduddin *et al.* 2021). Phytochemical investigations have revealed that C. esculenta contains a diverse range of secondary metabolites, including phenolic acids, flavonoids, tannins, alkaloids, and saponins, which contribute to its significant pharmacological potential. These compounds are responsible for the plant's antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and hepatoprotective properties (Rungsung et al. 2015). Among these bioactive constituents, gallic acid (3,4,5-trihydroxybenzoic acid) is one of the most important phenolic compounds. It is widely recognized for its strong antioxidant, anti-inflammatory, anticancer, and antimicrobial activities, and serves as a key marker compound in the standardization of herbal extracts.

The quantification of gallic acid in plant materials is essential for evaluating their pharmacological potential and ensuring quality control in herbal formulations. Although several analytical techniques such as High-Performance Thin Layer Chromatography (HPTLC) has emerged as a preferred method due to its simplicity, precision, cost-effectiveness, and capability for simultaneous analysis of multiple samples. HPTLC provides accurate qualitative and quantitative data with enhanced resolution and sensitivity, making it suitable for rapid phytochemical profiling and standardization of herbal drugs.

Despite extensive studies on the nutritional and medicinal attributes of C. esculenta, limited information is available on the distribution and quantification of gallic acid across its different parts. Hence, the present study aims to develop and validate a simple, accurate, and reliable HPTLC method for the qualitative and quantitative estimation of gallic acid from the extract of Colocasia esculenta (L.). This research not only contributes to the phytochemical characterization of C. esculenta but also supports its potential use in nutraceutical and pharmaceutical applications by providing a foundation for quality control and standardization.

Materials and Methods

2.1 Plant Material Collection and Preparation

Fresh and healthy samples of *Colocasia esculenta* (L.) were collected from agricultural fields. The plant material was washed thoroughly under running tap water to remove soil and debris, followed by rinsing with distilled water (Dolker *et al.*, 2024). Samples were shade-dried for 7–10 days at room temperature, then ground into a fine powder using a mechanical grinder. The dried powder was stored in airtight containers for further analysis (Rungsung *et al.* 2015).

2.2 Extraction of Colocasia esculenta

After dry to mixing sample then the dried sample powder (5.0 gm) was weighed, placed in a cheese cloth and kept in thimble; Methanol solvent used for extraction. Solvent was added to a round bottom flask, which was attached to a Soxhlet extractor and condenser. The crushed plant seed (material) was loaded into the thimble, which was then placed inside the Soxhlet extractor (Lewu *et al.* 2009). The solvent was heated using the heating mantle as the solvent boils, vapour starts to rise to extraction chamber, moving through

the apparatus to the condenser (Kumar *et al.*, 2023). The condensate then drips into the reservoir containing the thimble. Once the level of solvent reached the siphon it was poured back into the flask and the cycle begins again. The process ran for a total of 6 hours (Kumar *et al.* 2023). The collected extract was concentrated and sifted through Whatman no. 41 channel paper. All the concentrates (Methanol) were exposed to Qualitative tests for the distinguishing proof of different phytochemical constituents according to standard methods according to (Priyanka *et al.* 2020).

Determination of Extraction Yield

The extraction yield (%): = weight of extract after evaporation solvent and freeze drying/ dry weight of the sample × 100

2.3 Chemicals and Reagents

All chemicals and solvents were of analytical reagent grade and obtained from Merck (India). Standard **gallic acid** (purity ≥99%) was procured from Loba Chemie Pvt. Ltd., Mumbai, India. Methanol, toluene, ethyl acetate, and formic acid were used for mobile phase preparation. **Precoated silica gel 60 F_{254} HPTLC plates** (E. Merck, Darmstadt, Germany; 10 × 10 cm, 0.2 mm thickness) served as the stationary phase (Srivastava et al. 2011).

2.4 Preparation of Standard Solution & sample

A standard stock solution of gallic acid was prepared by dissolving 10 mg of pure gallic acid in 10 mL of methanol to obtain a concentration of 1000 µg/mL. Working standard solutions were freshly prepared by serial dilution in methanol for application on the HPTLC plate (Adhikari *et al.* 2023). Accurately weighed 100 mg of the methanolic extract of *C. esculenta* was dissolved in 1 mL of methanol, sonicated for 10 min to ensure complete dissolution, and filtered through a 0.45 µm syringe filter before application on the plate (Adhikari *et al.* 2023).

2.5 High-Performance Thin-Layer Chromatography (HPTLC) Method for Qualitative and Quantitative

High-Performance Thin-Layer Chromatography (HPTLC) was conducted using pre-coated silica gel 60 F_{254} HPTLC plates (50 × 100 mm; Merck, Darmstadt, Germany) as the stationary phase. Samples of the test substance (labelled KA1) were prepared in ethanol and applied to the plate in varying volumes (5.0, 6.0, 8.0, and 9.0 µL) using a CAMAG Linomat 5 automatic applicator (Serial No. 100632, CAMAG, Muttenz, Switzerland). Sample bands were applied 8.0 mm from the lower edge of the plate, with a band length of 8.0 mm and an inter-track distance of 10.4 mm (Mandrioli *et al.*, 2019). The dosage speed was set at 100 nL/s, and a pre-dosage volume of 0.20 µL was used to ensure accurate sample delivery.

Chromatographic development was performed in a twin-trough glass chamber (20 × 10 cm) pre-saturated for 20 minutes with a mobile phase as mentioned in the report. Saturation was carried out using appropriate filter paper liners to ensure consistent vapor phase conditions. Plates were developed up to a solvent front distance of 70 mm, followed by drying at ambient room temperature for 5 minutes.

Densitometric evaluation of the plates was conducted using a CAMAG TLC Scanner 3 (Serial No. 140507), operated under visionCATS software version 3.2.23095.1 (CAMAG, Switzerland). Scanning was performed in absorbance mode at multiple wavelengths 254 nm, 366 nm, 430 nm, and 480 nm using deuterium and tungsten lamps. The scanner was configured with automatic detector mode, a scanning speed of 20 mm/s, a resolution of 100 µm/step, and a slit dimension of 5 × 0.45 mm (micro).

Data acquisition and processing included Savitzky–Golay smoothing (window size 7), baseline correction using the lowest slope method, and peak detection using a Gaussian legacy algorithm with sensitivity set to 0.1, peak separation of 1, and a threshold of 0.1. Integration was carried out over an RF range of 0.00 to 1.00. Each sample volume yielded consistent peak patterns with reproducible RF values, supporting the robustness of the method. All operations were executed in accordance with CAMAG standard practices and analytical guidelines for HPTLC (CAMAG; Sethi, 1996; WHO, 2023).

3. Results and Discussion

3.1 HPTLC Analysis of Gallic Acid

A validated High-Performance Thin-Layer Chromatography (HPTLC) method was successfully developed for the qualitative and quantitative estimation of gallic acid in the methanolic extract of *Colocasia esculenta* (L.). The analysis was performed on silica gel 60 F_{254} plates using a mobile phase consisting of **toluene: ethyl acetate: formic acid (5:4:0.2, v/v/v)**. The developed chromatogram showed a distinct, well-resolved spot for gallic acid at an **Rf value of 0.51 ± 0.02**, which was comparable to that of the standard gallic acid (Rf = 0.515). This confirmed the presence of gallic acid in the plant extract (Figure 1). The plates were scanned at **254 nm**, where gallic acid exhibited a sharp and compact peak without interference from other phytoconstituents, indicating the specificity of the developed method.

3.2 Calibration Curve and Quantitative Estimation

The calibration curve was constructed using standard gallic acid at three concentrations corresponding to applied volumes of 4, 5, and 6 µL. The corresponding peak areas were 0.05230, 0.06540, and 0.08050 AU, respectively (Table 1). A linear relationship was observed between peak area and concentration within the applied range, following the regression equation:

$$Y=1.41x-44.333Y$$

where Y represents the peak area and x denotes the concentration (ng/spot). The correlation coefficient (R^2 = 0.998) demonstrated excellent linearity of the calibration curve (Figure 2).

Using the above equation, the concentration of gallic acid in the methanolic extract of *Colocasia esculenta* was calculated to be 357 ng/spot. This quantification confirms that gallic acid is a significant phenolic component present in the extract.

Table 3.2 [A]: Linear regression data for standard gallic acid 254nm.

Sr.No	Concentration (ng)	Volume (µL) Gallic acid	Rf	Peak Area (AU)	Regression Equation	Correlation Coefficient (R^2)
1	400	4	0.539	0.0523	Y = 1.41x − 44.333	0.998
2	500	5	0.539	0.0654		
3	600	6	0.539	0.0805		

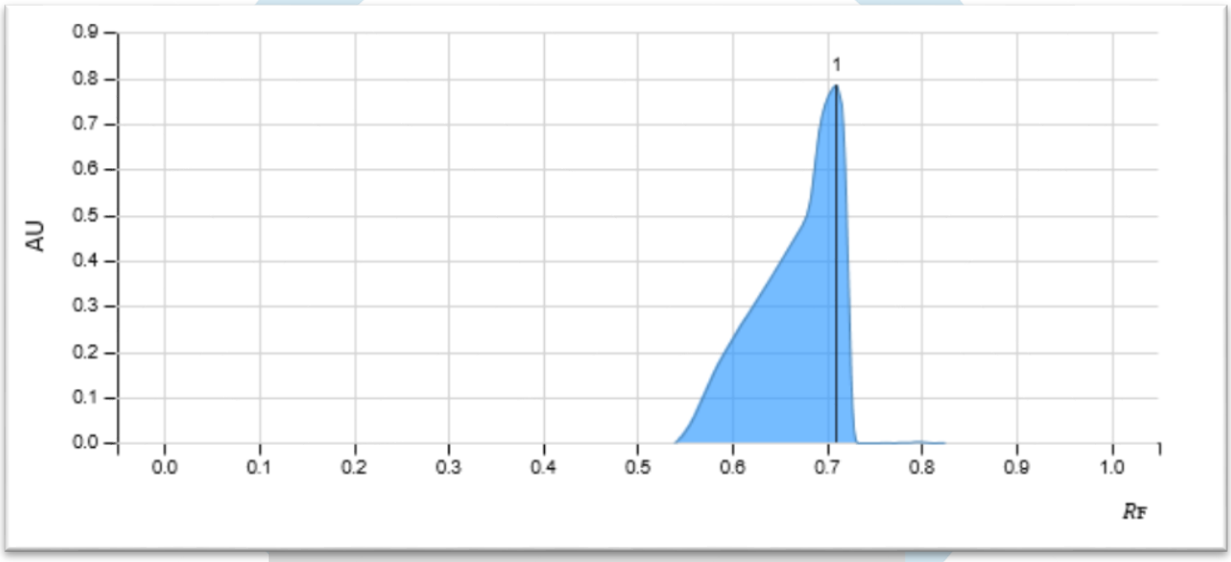


Fig 3.2 [A]: Densitometric HPTLC chromatogram showing the peak corresponding to gallic acid (400 ng) at Rf ≈ 0.69 for quantification in plant extract.

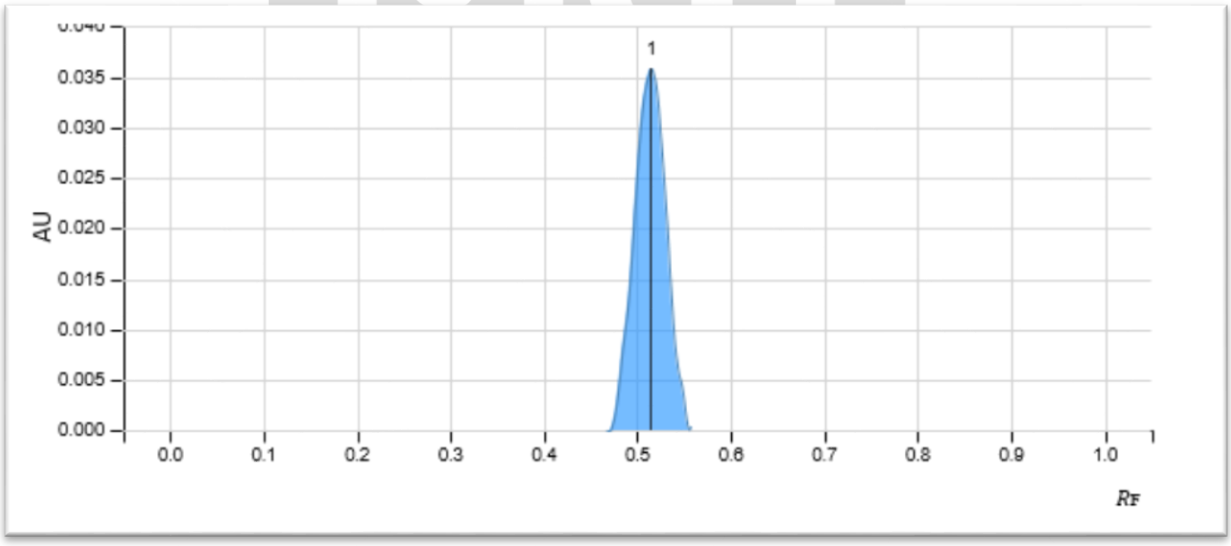


Fig 3.2 [B]: Densitometric HPTLC chromatogram showing the peak corresponding to gallic acid (500 ng) at Rf ≈ 0.69 for quantification in plant extract.

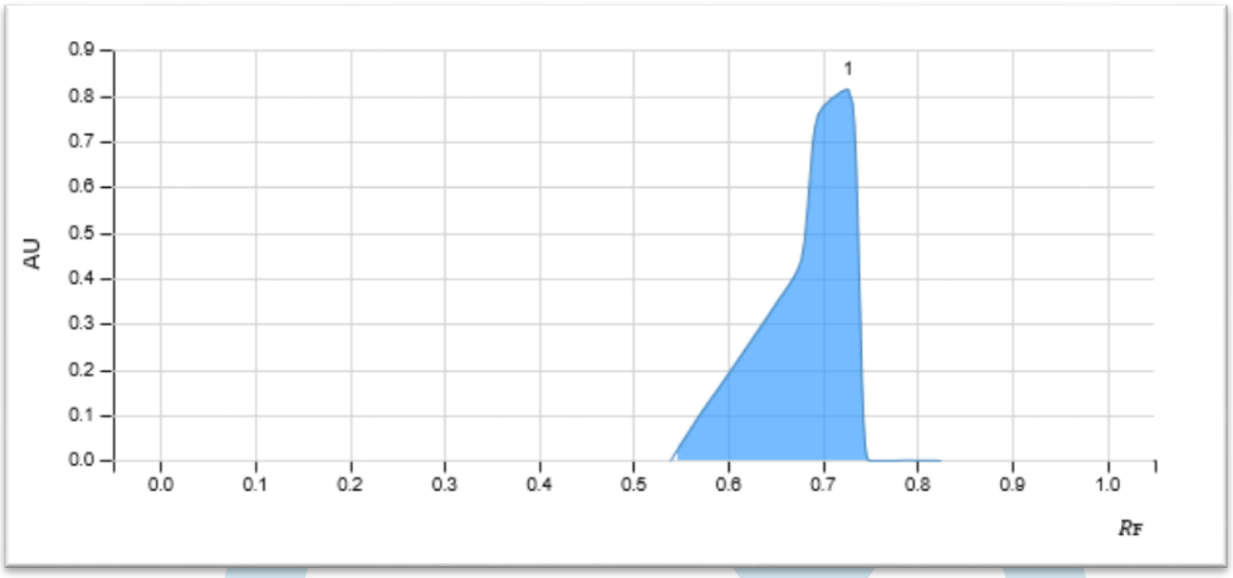
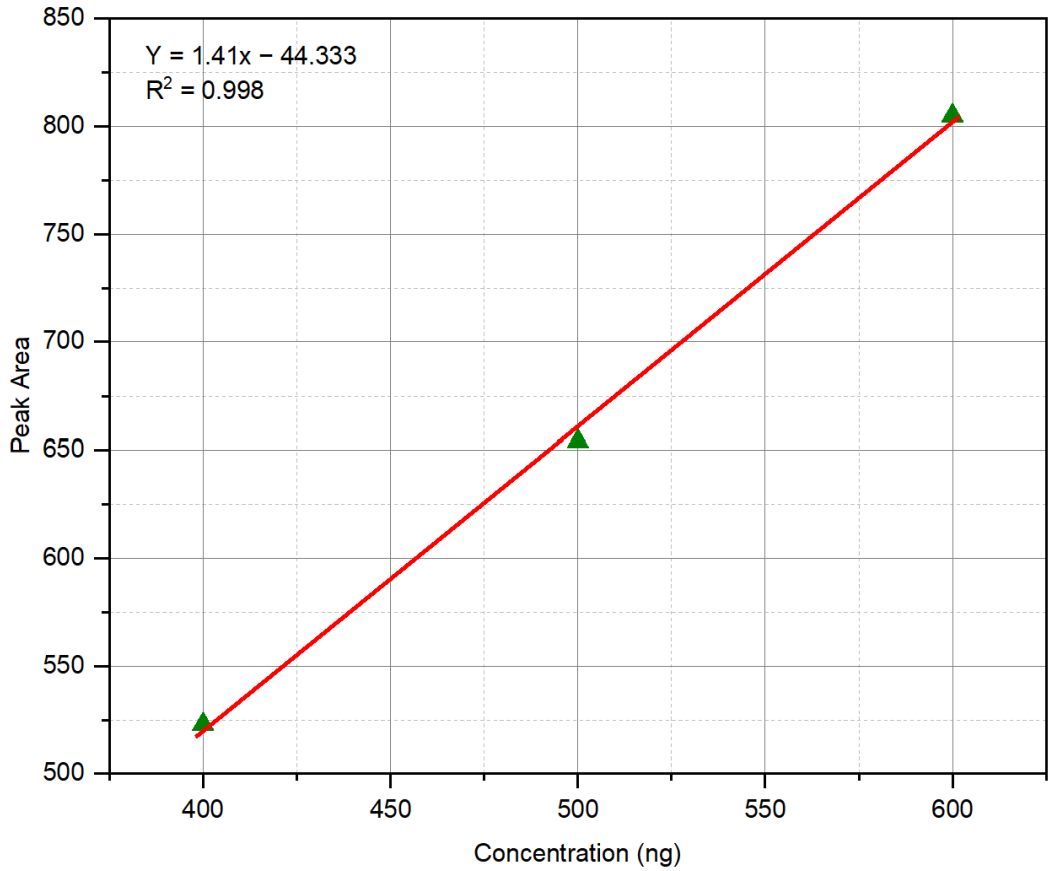


Fig 3.2 [C]: Densitometric HPTLC chromatogram showing the peak corresponding to gallic acid (600 ng) at Rf ≈ 0.69 for quantification in plant extract.

Area calibration for substance Gallic acid @ 254 nm:



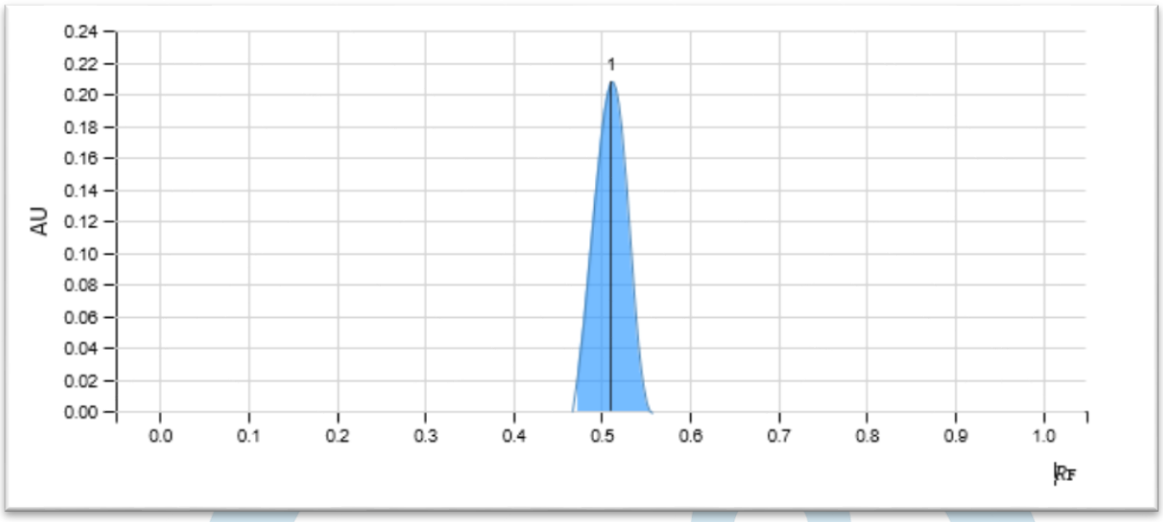


Fig 3.2 [D]: Densitometric HPTLC chromatogram showing the peak corresponding to gallic acid at Rf ≈ 0.69 for quantification in plant extract

Table 3.2 [B]: Quantitative estimation of gallic acid in *Colocasia esculenta* extract

Sample	Volume (µL) Gallic acid	Peak Area (AU)	Calculated Concentration (ng/spot)
C. esculenta methanolic extract	5	0.046	357 ng/spot

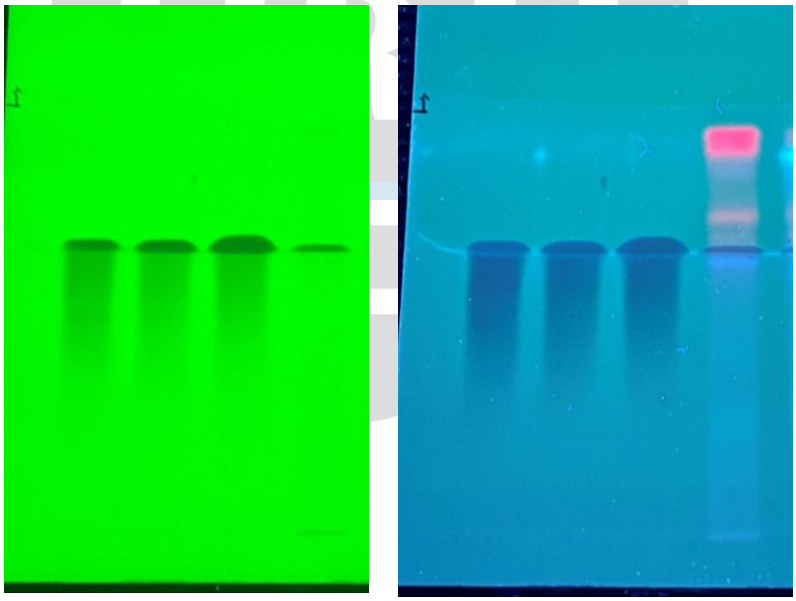


Fig 3.3 [E]: HPTLC chromatogram for quantification of gallic acid in plant extract under visible light (left) and UV light at 366 nm (right). Tracks represent standard gallic acid and sample extracts showing corresponding bands indicating the presence of gallic acid

3.3 Discussion

Gallic acid is a well-known polyphenolic compound with remarkable antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. The present study successfully confirmed its presence in *Colocasia esculenta*, a plant already recognized for its nutritional and medicinal importance. The distinct chromatographic separation and consistent peak response indicate that the developed HPTLC protocol is reliable for both qualitative and quantitative analysis.

The observed concentration of **357 ng/spot** highlights that *C. esculenta* contains a measurable amount of gallic acid, which contributes to its reported pharmacological activities. The rhizomatous tissues are likely to serve as the primary storage sites for phenolic compounds, as seen in similar studies on *C. esculenta* and other medicinal aroids.

These findings align with earlier reports by Adhikari and Saha (2025), who developed an HPTLC method for the estimation of quercetin in various parts of *C. esculenta*, demonstrating that such chromatographic approaches are efficient tools for identifying and quantifying bioactive compounds in plant matrices. The present HPTLC method, with its short analysis time, simple solvent system, and high accuracy, can serve as a valuable analytical technique for the standardization and quality control of herbal preparations containing *C. esculenta*. (Geneva *et al.* 2005).

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. Gallic acid 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) (Adhikari *et al.* 2023). This research contributes valuable information on the Gallic acid content of different parts of C. esculenta (L.) parts, potentially paving the way for further exploration of its potential health benefits and applications.

Acknowledgment

The authors would like to sincerely acknowledge the support and facilities provided by the CytoGene Research and Development, Lucknow. We are grateful for their assistance in carrying out the experiments and providing the necessary resources, which greatly contributed to the successful completion of this study.

Reference

- Arulmozhi S, Mazumder PM, Ashok P, Narayanan SL. Pharmacological activities of Alstonia scholaris Linn. (Apocynaceae). Pharmacognosy Reviews Phcog. Rev. 2007; 1(1):163-170.
- Rungsung W, Ratha KK, Dutta S, Dixit AK, Hazra J. Secondary metabolites of plants in drug discovery. World Journal of Pharmaceutical Research World J. Pharm. Res. 2015; 4(07):604-613.
- Li HM, Hwang SH, Kang BG, Hong JS, Lim SS. Inhibitory effects of C. esculenta (L.) Schott constituents on aldose reductase. Molecules. 2014; 19(9):13212-13224. https:// doi.org/10.3390/molecules190913212
- Asaduddin, A. H., Maulani, U. N., Sari, A. Y., Hawari, K., & Ayusari, A. A. (2021, April). Taro ice cream: addition of Colocasia esculenta stem to improve antioxidant activity in ice cream. In *IOP Conference Series: Materials Science and Engineering* (Vol. 1143, No. 1, p. 012038). IOP Publishing.
- Sethi, S. (2024). Building an Operations Management Department from Nowhere to Here (1996-2023): Anecdotes and Lessons Learned. *Available at SSRN 4831570*.

- Priyanka, K. and Ahmad, M.S., 2020. Phytoconstituents characterization of extricates prepared from seeds of Hallucinogenic plants. Indian J. Applied & Pure Bio. Vol, 35(2), pp.195-204.
- Lewu MN, Adebola PO, Afolayan AJ. *Effect of cooking on the proximate composition of Colocasia esculenta (L.) Schott tubers.* Int J Food Sci Nutr. 2009;60(Suppl 4):81–86.
- Rungsung W, Ratha KK, Dutta S, Dixit AK, Hazra J. *Secondary metabolites of plants in drug discovery.* World J Pharm Res. 2015;4(7):604–613.
- Srivastava M. *High-Performance Thin-Layer Chromatography (HPTLC).* Springer; 2011.
- Adhikari T, Saha P. *Development and validation of an HPTLC method for qualitative and quantitative estimation of quercetin in Glinus oppositifolius (L.).* Int J Pharm Sci Res. 2023;14(1):35–42.
- ICH. *Validation of Analytical Procedures: Q2(R1).* International Council for Harmonisation, Geneva, 2005.
- Dolker, D., Kaur, K., Behera, S., Nogia, P., Rawat, S., Kumari, V. and Pati, P.K., 2024. Harnessing the Potential of Plant Tissue Culture Techniques for the Herbal Industry. In In Vitro Propagation and Secondary Metabolite Production from Medicinal Plants: Current Trends (Part 1) (pp. 265-325). Bentham Science Publishers.
- Kumar, A., Siddiquee, A. and Ahmad, M.S., Extraction and characterization of bioactive components of Albizia lebbeck (L.) Benth., Cassia fistula Linn. and Delonix regia (Hook.) Raf. using GC-MS.
- Mandrioli, M., Tura, M., Scotti, S. and Gallina Toschi, T., 2019. Fast detection of 10 cannabinoids by RP-HPLC-UV method in Cannabis sativa L. Molecules, 24(11), p.2113.