Chemistry Africa (2023) 6:2871–2878
https://doi.org/10.1007/s42250-023-00714-3

ORIGINAL ARTICLE

Quantitative Assessment of an Antimalarial Flavonoid in the *Citrus maxima* (Burm.) Merr. Hydroalcoholic Root Extract using HPTLC Densitometric Analysis

Neelutpal Gogoi[1] · Ashis Kumar Goswami[2] · Dipak Chetia[1]

Received: 3 April 2023 / Accepted: 31 May 2023 / Published online: 15 June 2023

Abstract

Purpose The main purpose of the study was to standardize the hydroalcoholic (1:1) root extract (CM-HA) of *Citrus maxima* (Burm.) Merr using luteolin, a moderately powerful antimalarial flavonoid.

Methods The quantitative estimation of phenolic and flavonoid contents were done using standard methods. The qualitative and quantitative characterization of CM-HA was also carried out using High Performance Thin Layer Chromatography (HPTLC) based densitometric analysis using a validated method.

Results The quantities of phenolic and flavonoid contents were found to be 68.83 ± 1.83 mg gallic acid equivalent per gm and 40.04 ± 0.97 mg quercetin equivalent per gm of CM-HA. In HPTLC analysis, the marker compound (luteolin) was separated using a solvent system made up of toluene, ethyl acetate, and formic acid (10:8:1 v/v/v). Luteolin was quantified using densitometric scanning under a deuterium lamp at a 350 nm wavelength in absorbance mode. With the ideal solvent system, this validated approach produced a compact band of luteolin at an R_f value of 0.532 ± 0.029. Before quantitative estimation, the created method's precision, accuracy and reproducibility were verified as per the guideline of the International Council on Harmonization (ICH). For luteolin, linear results were seen graphically with a correlation coefficient of 0.990322 in the 400–1400 ng/band range. It was discovered that luteolin had detection and quantification limits of 30 and 80 ng/band, respectively. Eventually, it was discovered that there were 7.203 ± 0.557 mg of luteolin per 1 g of CM-HA.

Conclusion The study confirmed the presence of the antimalarial flavonoid luteolin in sufficient amount in the root of the plant.

Keywords *Citrus maxima* (Burm). Merr. · Luteolin · High Performance Thin Layer Chromatography · Antimalarial

Abbreviations

CM-HA	Citrus maxima hydroalcoholic extract
CV	Coefficient of variation
HPTLC	High performance thin layer chromatography
ICH	International Council on Harmonization
LOD	Limit of detection
LOQ	Limit of quantification
R_f	Retention factor
TLC	Thin layer chromatography

1 Introduction

Northeast India is a biodiversity hotspot region with varieties of natural resources having use in traditional practices for the treatment of different diseases and disorders. The local people of these regions utilized these resources, especially some plants, to prepare traditional herbal remedies [1]. People from rural areas are treated with these traditional remedies by local practitioners. *Citrus maxima* (Burm.) Merr. or Pomelo (Assamese: Robab Tenga) is such a wild plant that is reported to have many significant biological

✉ Neelutpal Gogoi
neelutpalgogoi@dibru.ac.in

[1] Department of Pharmaceutical Sciences, Faculty of Science & Engineering, Dibrugarh University, Dibrugarh, Assam, India

[2] Department of Pharmacognosy, Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati, Assam, India

activities like antioxidant [2], antibacterial [2], antidiabetic [3], hepatoprotective [4], anticancer [5], antidepressant [6] etc. During field survey, it was found that the plant's root part is being used to prepare an antimalarial herbal remedy. Along with root, the unripe fruit of the plant is also used in the treatment of fever associated with malaria. Later, in extensive literature review, the utilization of the different parts of the plant *C. maxima* in the treatment of malaria was reported [7]. In one of our previous studies, we found that the fruit part is rich in flavonoid compounds. Among the flavonoids, luteolin showed moderate activity against the chloroquine sensitive (3D7) and chloroquine resistance (RKL-9) strains of *Plasmodium falciparum* [8]. In another study, the hydroalcoholic root extract (CM-HA) of the plant was found to be potent (< 5 µg/ml) against both the 3D7 and RKL-9 strains of *P. falciparum* [9]. Later during thin layer chromatography analysis, the presence of luteolin in the CM-HA extract was detected. Hence, this study developed a method for quantitative estimation of luteolin in the CM-HA extract using high performance thin layer chromatography (HPTLC) technique. The HPTLC densitometric technique is a very simple and fast method for the quantitative estimation of marker compounds in plant-based samples [10, 11]. The developed method was validated according to International Council on Harmonization (ICH) guidelines; hence it can be utilized for quality control related analysis of the raw materials or any phytopharmaceuticals developed from the extract [12].

2 Methods

2.1 Instrumentation and Reagents

The analysis was conducted using TLC aluminium plates (20 cm × 10 cm and 10 cm × 10 cm) precoated with silica gel 60 F_{254} (Merck) (0.2 mm thickness). Using a 100 µl syringe (Hamilton), spotting was done with the CAMAG Linomat V semiautomatic Sample Spotter (CAMAG Muttenz, Switzerland). Based on the size of the aluminium plates, the different CAMAG glass twin trough chambers (20 cm × 10 cm and 10 cm × 10 cm) were used for development. Using visionCATS 2.5 software, a CAMAG TLC Scanner IV carried out the densitometric study. After development, the plates were examined in a CAMAG UV cabinet (254 and 366 nm).

The analytical-grade solvents from Merck included ethanol, methanol, toluene, ethyl acetate, and formic acid (Mumbai, India). Merck supplied the analytical-grade aluminium TLC plates that were coated with Silica Gel 60 F_{254} (Mumbai, India). We bought luteolin (97%), an analytical-grade marker compound, from Alfa aesar (Thermo Fisher), India. Quercetin (≥ 95%) and Gallic acid (≥ 97%) were purchased from Sigma-Aldrich, India.

2.2 Plant Material

In February 2021, the root of *C. maxima* was collected from the wild sources in the Longpotia region of Charaideo District, Assam. The plant was identified and authenticated by the Botanical Survey of India, Shillong, India. The coarsely powdered single batch plant materials were extracted using a hydroalcoholic solvent system (1:1) for 72 h by cold maceration [13]. The solvent was evaporated using a rotary vacuum evaporator (IKA Rotary Evaporator RV 8 V) and finally dried by lyophilization technique (IIC Industrial Corporation). The extract was kept in an airtight container in 4°C for future use.

2.3 Total Phenolic Content and Total Flavonoid Content

The total phenolic content (TFC) of the extract was determined utilizing the Folin-Ciocalteu reagent with gallic acid as the standard using spectrophotometric method [14]. The absorbance was measured at a wavelength of 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800) and content was expressed as a milligrams of gallic acid equivalents (GAE) per gram of dry extract weight.

The total flavonoids content (TFC) of the extract was determined by the aluminium chloride based colorimetric method with quercetin as the standard [15]. The absorbance was measured at a wavelength of 415 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800) and the content was expressed as a milligrams of quercetin (QE) equivalents per gram of dry weight. All samples were analysed in triplicate to ensure accuracy and reliability of the results.

2.4 Preparation of Standard and Test Sample

In a volumetric flask, 10 mg of luteolin were dissolved in 10 ml of methanol to create 1 mg/ml of luteolin stock solution. 200 mg of the CM-HA extract was dissolved in 10 ml of methanol to create the test sample with a 20 mg/ml concentration. Then, for ten minutes, the test and standard samples were sonicated (Ultrasonic cleaner STD Rivotek, India). Before being used in the analysis, the samples were centrifuged (RM-03 Plus, REMI, India) for 15 min at 4000 rpm following sonication [16].

2.5 TLC Fingerprint and Chromatography

2.5.1 Chromatographic Conditions

The experiment was conducted at 25 ± 2 °C and 55 ± 3% relative humidity. The slit size was held constant at 5 mm × 0.2 mm, and 20 mm/s was used for the scanning speed [17]. For chromatography, luteolin and CM-HA extract were added in triplicate on a silica gel 60 F_{254} coated TLC aluminium plate. Depending on the plate size, either 10 ml or 20 ml of mobile phase were used for each chromatography.

2.5.2 Qualitative Fingerprint Analysis and Optimization of Mobile Phase

To achieve optimal separation with clear resolution, the marker compound and the test extract (CM-HA) were applied onto a TLC plate measuring 10 cm × 10 cm. A suitable mobile phase was employed to facilitate the separation process. The plate was subsequently examined using absorbance mode at 254 nm to visualize the distinct bands formed as a result of separation. Upon identifying the plate with satisfactory separation and resolution, it was scanned at 254 nm to generate a chromatogram.

2.5.3 Quantification of Luteolin using HPTLC Densitometric Technique

An accurate and easy approach was established for quantifying luteolin in the hydroalcoholic extract of *C. maxima* roots since there was no previous report of luteolin quantification in *C. maxima* using HPTLC. Toluene, ethyl acetate, and formic acid were used as the mobile phase in a 10:8:1 ratio during the optimization of the TLC process. Following sample application, the plates were saturated with the mobile phase (10 ml or 20 ml) for 20 min before being developed in CAMAG twin trough glass tanks. At room temperature, the plates were developed to a height of 7 cm. In order to acquire the maximum quality of the sample and reference in their natural state, derivatization was skipped after development because dark brown bands had developed on the plates. By scanning the plates at 350 nm in absorbance mode, the CAMAG TLC Scanner IV quantitatively analyzed the chemicals. The identification of luteolin was verified by superimposing the UV spectra of the samples and standards within the same Retention factor (R_f) window.

2.5.4 Calibration Curve of Luteolin

A calibration curve employing a reference luteolin and a concentration range of 400–1400 ng/band was used to ascertain the luteolin content. Standard luteolin (1 mg/ml) was produced as a stock solution in methanol. Using a semi-automatic sample spotter, various stock solution volumes (0.4, 0.6, 0.8, 1.0, 1.2, and 1.4) were spotted on TLC plates to produce concentrations of 400, 600, 800, 1000, 1200, and 1400 ng/band, respectively (band width of 8 mm, distance between tracks 11.8 mm).

2.5.5 Validation of HPTLC Method

According to ICH guidelines, the method developed for luteolin identification and quantification in the CM-HA extract was verified for repeatability, precision, and accuracy [8, 16, 18].

2.5.5.1 Precision The precision, reproducibility, and accuracy of the developed method were assessed for the analytical method's validation in accordance with ICH recommendations. The instrument precision was evaluated using replicate luteolin solutions (n = 6). To assess the intraday precision, replicate administrations of newly made luteolin solution at the same concentrations (400–1400 ng/band) were used at six different time points (n = 6). Intermediate precision was ascertained by analyzing repeated (n = 6) applications of luteolin solution at the same doses (400–1400 ng/band) on six different days. To indicate the repeatability of the method, the results were represented as a percentage coefficient of variation (% CV) of the peak area.

2.5.5.2 Repeatability The reproducibility of the method was confirmed by testing 1000 ng/band of luteolin on a TLC plate (n = 10). The results were given as a percentage CV.

2.5.5.3 Limit of Detection and Limit of Quantification Several concentrations of the luteolin standard solutions were used, together with methanol as a blank, to evaluate the limits of detection and quantification. The results were calculated using the signal to noise (S/N) ratio. Limits of quantification (LOQ) and detection (LOD) were established at S/N ratios of 10:1 and 3:1, respectively.

2.5.5.4 Selectivity The selectivity of the procedure was determined by examining standard luteolin and CM-HA extract. By comparing the R_f value and the spectra of the band of the standard with those of the sample, it was possible to identify the band for luteolin in the sample. By examining the spectra at three different levels, namely the band's peak start, peak middle, and peak end positions, it was possible to determine the peak purity of luteolin.

2.5.5.5 Robustness Within the tolerance of 10%, the mobile-phase volume, mobile-phase composition, and mobile-phase saturation time were all calculated. The % CV

between the data for each variable condition was computed to get the results.

2.5.5.6 Accuracy To check the accuracy of the experiment using the conventional addition approach, the recovery experiment was conducted at three levels: 50%, 100%, and 150% addition of luteolin. By spiking, luteolin was added at a rate of about 500 ng/band. Calculations were made to determine the percentage recovery values and average percentage recovery values for luteolin.

2.5.5.7 System Suitability The system suitability tests were conducted to confirm the experiment's repeatability and resolution. Using the same chromatographic parameters, a freshly made standard solution of luteolin was applied at a concentration of 1000 ng/band (n = 6), and the densitograms were then obtained by scanning.

3 Results

3.1 Total Phenolic and Flavonoid Contents

The yield of the extract (CM-HA) was found to be 8.305% w/w in comparison to the raw plant material taken for the extraction process. The different plant secondary metabolites present in the extract were already reported in our previous publication [9]. The *Citrus* plants contains sufficient amounts of flavonoids which possess significant antimalarial activity [19]. Hence, here we determined the equivalent quantity of phenolic and flavonoid contents which were found to be 68.83 ± 1.83 mg gallic acid equivalent per gm and 40.04 ± 0.97 mg quercetin equivalent per gm of raw extract respectively. The calibration curves of the standards are given in supplementary materials (Fig. S1 and Fig. S2).

3.2 TLC Fingerprint and Chromatography

After developing the plates up to a height of 7 cm, the R_f values and colour of the resolved bands were observed. Under 254 nm in absorbance mode, the bands of luteolin were discovered to be dark brown in colour. It was discovered that the luteolin band's R_f was 0.532 ± 0.029. The image of a TLC plate showing luteolin and CM-HA extract in absorbance mode at 254 nm is shown in Fig. 1. Luteolin's R_f value coincided with the R_f value of the band observed in CM-HA extract, which was determined to be in the range of 0.532 ± 0.029.

3.3 TLC Densitometric Quantification of Luteolin Using HPTLC

The chosen solvent system provided clear peak resolution. The band at R_f value 0.532 ± 0.029 was identified as luteolin using the chromatogram and UV spectra between 190 and 450 nm (Figs. 2 and 3). The spectra of CM-HA extract's corresponding band at wavelengths between 190 and 450 nm matched the spectra generated for luteolin, proving that luteolin was present in the sample. For the development of the TLC plates, the twin trough chamber was saturated for 20 min at room temperature. In a CAMAG visualization cabinet, the TLC plates were observed at 254 and 366 nm. As a result of the marker compound's absorbance property, better visibility was obtained at 254 nm (Fig. 2). The spectra produced in the range of 190–450 nm in absorbance

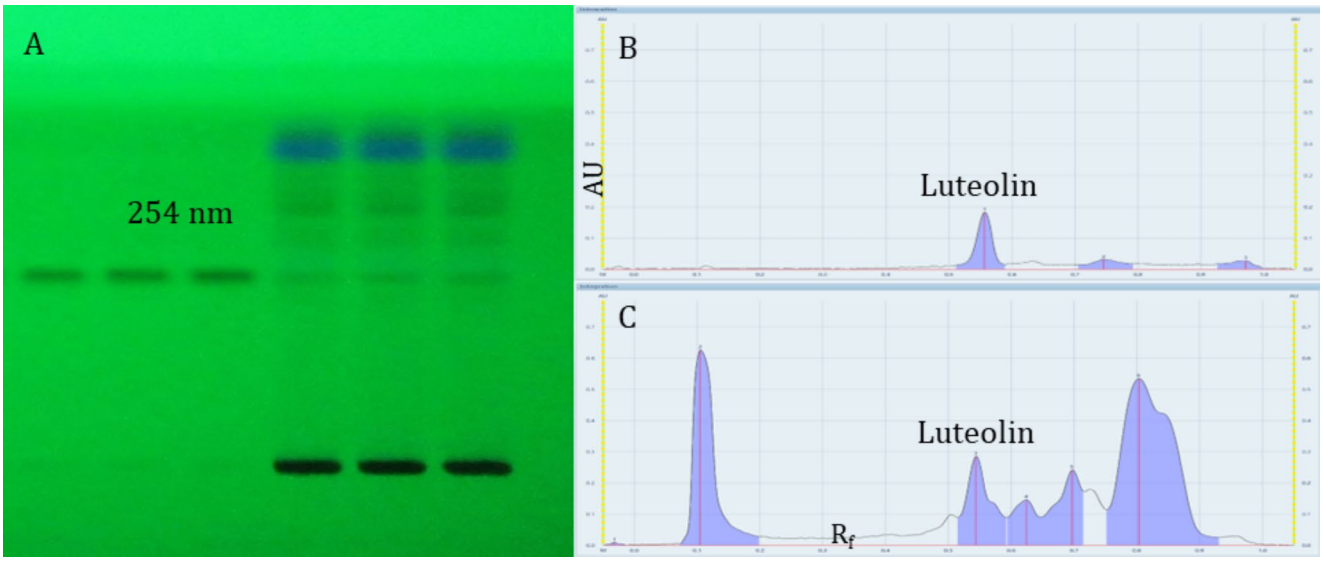

Fig. 1 Qualitative fingerprint analysis of CM-HA in presence of luteolin; (A) TLC plates observed under 254 nm in absorbance mode, (B) Chromatogram of luteolin and (C) Chromatogram of CM-HA extract

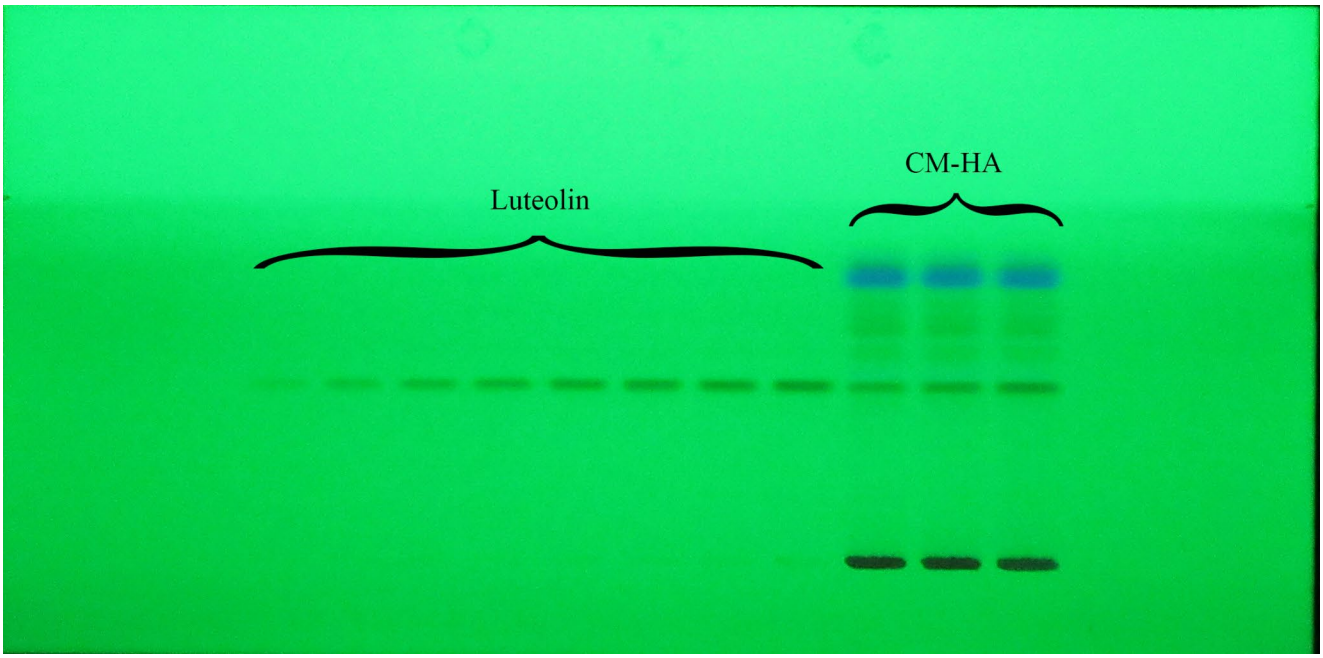

Fig. 2 Luteolin in different concentrations and CM-HA in triplicates for quantitative analysis

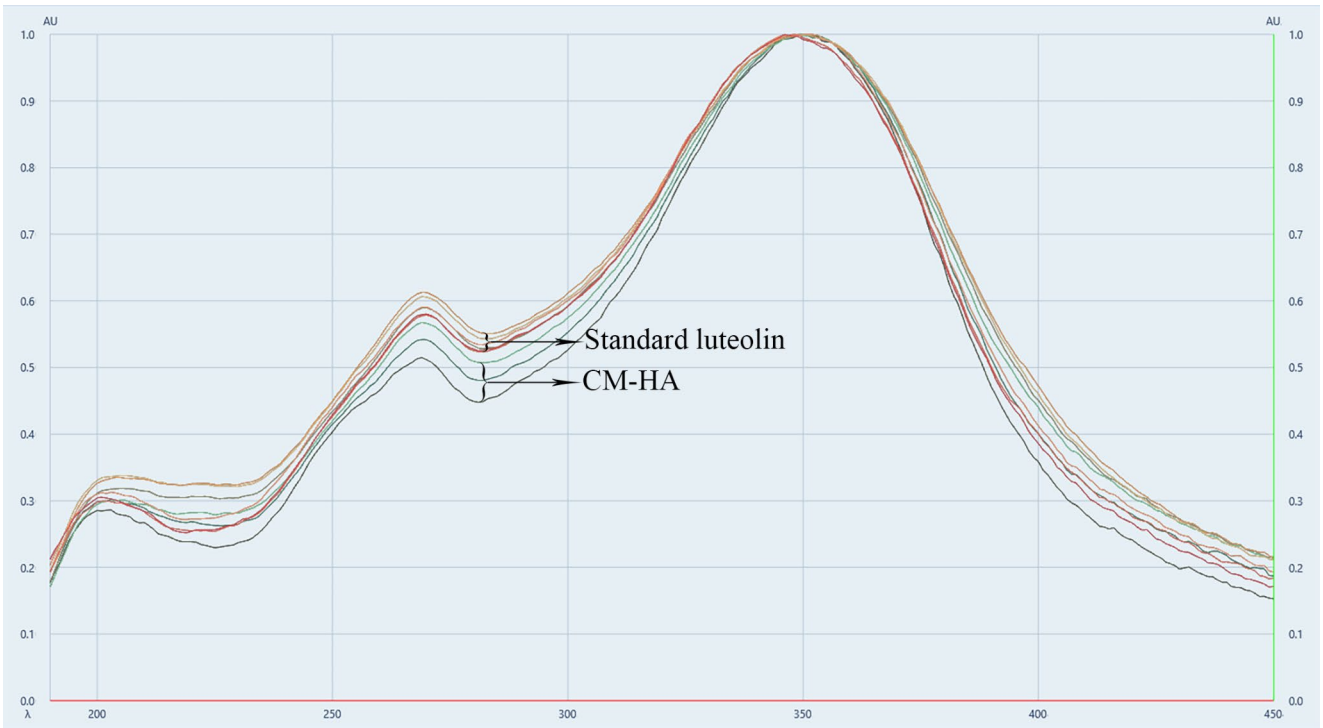

Fig. 3 Spectra obtained from the band of standard luteolin and CM-HA extract within the 190–450 nm wavelength range

mode revealed that the maximum absorbance of the luteolin was shown at 350 nm (Fig. 3). The presence of luteolin was established by comparing the densitometric chromatogram produced from the CM-HA extract with that produced from the luteolin at 350 nm in absorbance mode (Fig. 4). The R_f value for the peak corresponding to luteolin in the CM-HA extract was the same as for the standard luteolin (R_f 0.532 ± 0.029).

The equation for the linear regression line was $y = 1.694 \times 10^{-7}x + 1.946 \times 10^{-1}$. The standard curve's linear regression coefficient (R) was determined to be 0.99032. The regression data showed a solid linear association for

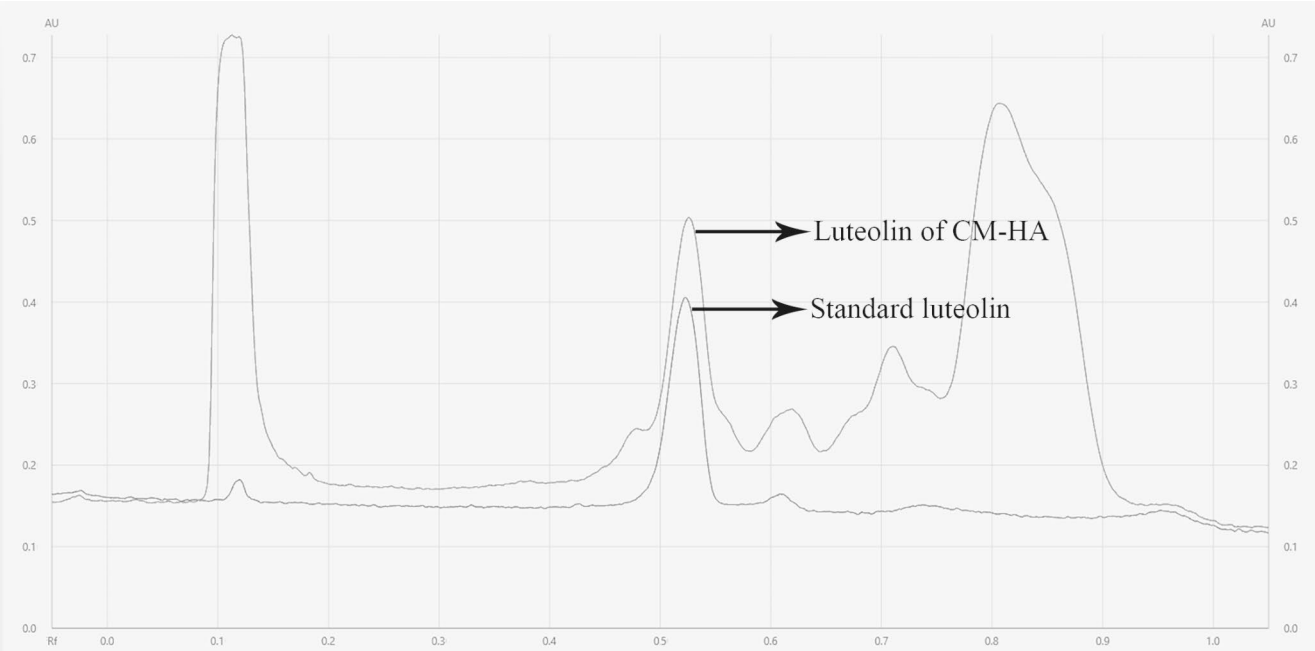

Fig. 4 Chromatogram of luteolin and CM-HA at 350 nm in absorbance mode

Table 1 Method development parameters for the quantification of luteolin in the CM-HA extract of *Citrus maxima* (Burm.) Merr

Parameters	Acceptance criteria	Results
Selectivity	-	Selective
Linear range (ng/band)	Linear, accurate & precise	400–1400 ng/band
Correlation coefficient	0.9–1.1	0.99032
Linear regression equation	-	$Y = 1.694 \times 10^{-7}x + 1.946 \times 10^{-1}$
LOD (ng/band)	-	30 ng/band
LOQ (ng/band)	-	80 ng/band
Percentage recovery	90–110%	94.35
Repeatability % CV, n = 10)	%CV ≤ 2	Complies
Precision (%CV)	%CV ≤ 2	Complies
Intraday (n = 6)	%CV ≤ 2	Complies
Interday (n = 6)	%CV ≤ 2	Complies

Table 2 Recovery studies of luteolin at 50%, 100% and 150% spiking by TLC Densitometric Method

Concentration of luteolin (ng/ml)		Area spotted		Total area (Sample + standard)	Total area obtained	% Recovery
Sample content	Spiked amount	Sample area	Spiked area			
100	50	2351	1698	4049	3838	94.78
100	100	2349	2313	4662	4517	96.88
100	150	2361	2638	4999	4764	95.29
Average recovery (%)						95.65

the 400–1400 ng/band concentration range. High correlation coefficient values confirmed the system's adherence to Beer's law and the linearity of calibration graphs. In the supplementary data, the calibration curve for standard luteolin is illustrated (Fig. S3). As indicated in Tables (1, 2 and 3), the TLC densitometric approach was validated in terms of precision, accuracy and repeatability. The percentage of luteolin recovered for the experiment was between 94.78 and 96.88%. The accuracy and repeatability of the approach were examined by measuring the peak area at six distinct concentration ranges. The results revealed low values of % CV (< 2%) for intraday (1.15–1.60%) and interday (0.37–1.51%) variation for various concentrations of luteolin (Table 3). These findings revealed that the approach had outstanding precision and reproducibility. For luteolin, the LOD value was determined to be 30 ng/band and the LOQ value to be 80 ng/band. The recovery investigations confirmed the study's accuracy because the

Table 3 Results of Interday and Intraday Precision of the Developed HPTLC Method (n = 6)

Luteolin (ng/band)	Intraday precision (% CV)	Interday precision (% CV)
400	1.186333	0.521881
600	1.152102	1.209253
800	1.274603	1.510421
1000	1.404733	0.663874
1200	1.601706	0.378669
1400	1.406598	0.834423

percentage recovery (90.74–96.47%) was discovered to be within allowable bounds. The low percentage CV (1.18%) demonstrated the robustness of the experiment and the reproducibility of the approach, demonstrating that luteolin remained stable throughout the analysis process. Based on this TLC densitometric analysis, the content of luteolin in the hydroalcoholic extract of the roots of *C. maxima* was found to be 7.203 ± 0.557 mg per 1 g of extract.

4 Discussion

The citrus plants are very much rich in phenolic and flavonoid contents specially the fruits. In this study we found the presence of both these two categories of compounds also in the root of *C. maxima* in significant quantities. Then we found the presence of luteolin in the qualitative TLC analysis of the extract. Luteolin is a moderately active antimalarial compound found in different plants. Luteolin is present in the fruit part of Citrus plants but in this study, we identified the presence of this compound in the root part of *C. maxima* plant [20]. Hence to quantify this compound in the hydroalcoholic extract of the root part, a simple and cost effective method was developed. The devised HPTLC method for luteolin determination was found to be exact, accurate, and selective. The optimized mobile phase gave good separation and resolution for luteolin in both the standard and the test extract. Fig. S4 displays the resolution of the separated luteolin peak in the test sample and the peak of the reference luteolin. The chromatogram of the hydroalcoholic extract with this mobile phase developed through densitometric HPTLC analysis can be employed as a standardized fingerprint for the quality assessment of the plant material. High correlation coefficient value (R = 0.99032) of the six-point calibration curve obtained from various concentrations indicated that the devised method had strong linearity and peak uniformity. The LOD and LOQ values showed that the new approach produced low noise levels. Consequently, within the specified experimental circumstances, this study establishes a novel, validated approach for quantification of luteolin and demonstrates that it can produce accurate results.

5 Conclusion

The root of the plant *C. maxima* is found to be rich in phenolic and flavonoid contents and we identified and quantified the presence of an antimalarial flavonoid, luteolin in the extract. The investigation showed that the suggested approach is selective for quantifying luteolin in herbal medication formulations and reproducible. The amount of luteolin determined by the verified approach is substantial for isolation as a pure chemical. This technique can be utilized proactively for quality control of raw pharmaceuticals containing luteolin in process and completed goods because it separates luteolin from other elements with good band resolution.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42250-023-00714-3.

Acknowledgements Authors are thankful to Dr. N. Odyuo, Scientist D & HoD, Botanical Survey of India, Eastern Regional Centre, Shillong, Meghalaya for authentication and identification of the plant sample.

Author contribution NG and DC had conceptualized and designed the work. NG performed the experimental work. NG and AKG evaluated the data. NG wrote the manuscript and DC reviewed the manuscript. All authors read and approved the final manuscript.

Funding The work was supported by University Grants Commission, New Delhi under UGC-SAP (DRS-I) [F.3–13/2016/DRS-I (SAP-II)].

Data Availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

References

1. Shankar R, Deb S, Sharma BK (2015) Traditional Healing Practices in North East India. Indian J Hist Sci 50(2):324–332. https://doi.org/10.16943/ijhs/2015/v50i2/48242
2. Dulay RM, Castro MEGD (2016) Antibacterial and antioxidant activities of three Citrus Leaves extracts. Der Pharma Lett 8(13):167–170
3. Muneer A, Shenoy A, Hegde K, Aamer S, Shabaraya AR (2014) Evaluation of the antidiabetic activity of Ethanolic Extract of *Citrus maxima* Stem Bark. Int J of Pharm Chem Sci 3(3):642–650
4. Chowdhury MRH, Sagar MAT, Tabassum N, Potol MA, Hosain H, Alam MA (2015) Supplementation of *Citrus maxima* Peel Powder prevented oxidative stress, fibrosis, and hepatic damage in Carbon Tetrachloride (CCl_4) treated rats. Evid Based Complementary Altern. https://doi.org/10.1155/2015/598179
5. KunduSen S, Gupta M, Mazumder UK, Haldar PK, Saha P, Bala A (2011) Antitumor Activity of *Citrus maxima* (Burm.) Merr. Leaves in Ehrlich's Ascites Carcinoma cell-treated mice. ISRN Pharmacol. https://doi.org/10.5402/2011/138737
6. Potdar VH, Kibile SW (2010) Evaluation of antidepressant-like effect of *Citrus maxima* Leaves in Animal Models of Depression. Iran J Basic Med Sci 14(5):478–483
7. Yetein MH, Houessou LG, Loubegnon TO, Teka O, Tente B (2013) Ethnobotanical study of medicinal plants used for the treatment of malaria in plateau of Allada, Benin (West Africa). J Ethnolpharmacol 146:154–163. https://doi.org/10.1016/j.jep.2012.12.022
8. Gogoi N, Chetia D, Gogoi B, Das A (2021) Multiple-targets Directed Screening of Flavonoid Compounds from Citrus Species to find out antimalarial lead with predicted Mode of Action: an in Silico and whole cell-based in vitro approach. Curr Comput Aided Drug Des 17(1):69–82. https://doi.org/10.2174/1573409916666191226103000
9. Gogoi N, Gogoi B, Chetia D (2021) In vitro antimalarial activity evaluation of two ethnomedicinal plants against chloroquine sensitive and resistant strains of *Plasmodium falciparum*. Clin Phytoscience 7(1):1–10. https://doi.org/10.1186/s40816-021-00269-1
10. Indrayanto G (2011) Analytical Aspects of High Performance Thin Layer Chromatography. In: Srivastava M (ed) High-performance thin-layer chromatography (HPTLC), 1st edn. Springer, Berlin, Heidelberg
11. Dhandhukia PC, Thakker JN (2011) Quantitative analysis and validation of Method using HPTLC. In: Srivastava M (ed) High-performance thin-layer chromatography (HPTLC), 1st edn. Springer, Berlin, Heidelberg
12. International Conference on Harmonization (ICH) (1994) ICH harmonised tripartite guideline. Validation of analytical procedures: Text and methodology Q2 (R1). In: Proceedings of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf Accessed 22 November 2022
13. Abubakar AR, Haque M (2020) Preparation of Medicinal plants: basic extraction and fractionation procedures for experimental Purposes. J Pharm Bioallied Sci 12(1):1–10. https://doi.org/10.4103/jpbs.JPBS_175_19
14. Wairata J, Fadlan A, Setyo Purnomo A, Taher M, Ersam T (2022) Total phenolic and flavonoid contents, antioxidant, antidiabetic and antiplasmodial activities of Garcinia forbesii King: a correlation study. Arab J Chem 15(2):103541. https://doi.org/10.1016/j.arabjc.2021.103541
15. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju YH (2014) Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. J Food Drug Anal 22(3):296–302. https://doi.org/10.1016/J.JFDA.2013.11.001
16. Goswami AK, Gogoi N, Shakya A, Sharma HK (2019) Development and validation of high-performance thin-layer Chromatographic Method for quantification of Berberine in Rhizomes of *Coptis teeta* Wall, an endangered species collected from Arunachal Pradesh, India. J Chromatogr Sci 57(5):411–417. https://doi.org/10.1093/chromsci/bmz009
17. Goswami AK, Gogoi N, Sharma HK (2022) Validated High-Performance Thin-Layer Chromatography-Mass Spectrometry Method and Stability Study of Linalool in the volatile oil of the Rhizomes of *Homalomena aromatica* Schott. J Chromatographic Sci. https://doi.org/10.1093/chromsci/bmac012
18. Stauffer M (2018) In: (ed) Calibration and validation of Analytical Methods - a sampling of current approaches. InechOpen
19. Rudrapal M, Chetia D (2016) Plant flavonoids as potential source of future antimalarial leads. Sys Rev Pharm 8(1):13–18. https://doi.org/10.5530/srp.2017.1.4
20. Tripoli E, Guardia ML, Giammanco S, Majo DD, Giammanco M (2007) Citrus flavonoids: molecular structure, Biological Activity and Nutritional Properties: a review. Food Chem 104(2):466–479. https://doi.org/10.1016/j.foodchem.2006.11.054