Int J Adv Life Sci Res. Volume 8(3) 83-93
https://doi.org/10.31632/ijalsr.2025.v08i03.007

International Journal of Advancement in Life Sciences Research

Online ISSN: 2581-4877

Journal homepage http://ijalsr.org

Original Article

Development and Validation of HPTLC Method for Estimation of Kaempferol and Luteolin in Arjunarishta Formulations Prepared by Traditional and Non-Traditional Mode

Atish Yadaorao Sahare*, Ravi Khatri

Institute of Pharmaceutical Science and Research, Sardar Patel University, Balaghat, Madhya Pradesh, India

**Corresponding Author's Email: atish.sahare@gmail.com*

Abstract

Arjunarishta is an *arishta* preparation that evolved through the utilisation of specific ingredients via fermentation. It safeguards and fortifies the circulatory system and optimises heart muscle function by influencing cholesterol levels and blood pressure. The analysis of kaempferol and luteolin in Arjunarishta formulations prepared by the traditional mode (AT), non-traditional modes (AI and AS), as well as in the commercial Arjunarishta version (AM), has been made reliable through the development of a simpler, error-free, and precise HPTLC approach. The isolated yeast species obtained from *Madhuca indica* flowers and the standard strain of *Saccharomyces cerevisiae* SC1011 were adopted to accelerate the fermentation rate in the non-traditional Arjunarishta formulations, AI and AS, respectively. The developed HPTLC approach has been evaluated with respect to linearity, robustness, ruggedness, quantification and detection limits, and accuracy. It was found that the levels of luteolin in AI, AS, AT, and AM were 0.285%, 0.267%, 0.135%, and 0.106% w/w, respectively, and the levels of kaempferol were 0.355%, 0.361%, 0.342%, and 0.224% w/w, respectively. This documented work is the first report on the HPTLC determination of luteolin and kaempferol in Arjunarishta. Numerous investigations have demonstrated the antioxidative and protective effects of these compounds on cardiovascular health. The rigorous qualitative and quantitative analysis of kaempferol and luteolin via HPTLC not only validates the presence and concentration of these compounds but also ensures the therapeutic efficacy and safety of Arjunarishta. This scientific validation is crucial for establishing Arjunarishta as a reliable formulation for managing disorders of the circulatory system.

Keywords: *Arjunarishta; HPTLC; Kaempferol; Luteolin*

Introduction

Ayurveda is among the globe's earliest and most comprehensive therapeutic approaches and has a long history of application in the healthcare sphere. Within this Vedic framework, there are multiple types of herbs and formulations made from them. In addressing several complex health concerns, Ayurvedic formulations feature an assortment of plants, each of which contains multiple potent phytochemicals that may work synergistically to deliver therapeutic benefits (Singh *et al*., 2025). Although there are numerous formulations in Ayurveda, fermented preparations such as *Arishta* and *Asava* represent distinctive modalities of treatment that set Ayurveda apart from other global medical systems. These aforementioned formulations have demonstrated efficacy in a variety of ailments affecting the body's entire systems (Tambe *et al*., 2023).

Arjunarishta is an arista conception that evolved by utilising specific ingredients through fermentation (Anonymous, 2008). It safeguards and fortifies the circulatory system and optimises heart muscle function by influencing cholesterol levels and blood pressure. *Terminalia arjuna, Vitis vinifera, Madhuca indica*, and *Woodfordia fruticosa* are steadily the indigenous constituents in this mixture. Air-dried

Received on :5th December 2024; Revised version received on :30th June 2025; Accepted: 3rd July 2025

Arjuna bark serves as the core element within Arjunarishta (Thakur, Patil & Gawhankar, 2020). It carries multiple important phytochemicals, covering flavonoids, phytosterols, glycosides, phenolics, tannins, and triterpenoids. Notably, luteolin and kaempferol are among the vital flavonoids detected in arjuna bark (Verma & Jogdand, 2021). Kaempferol's antioxidative attributes probably accounted for its functionality in sustaining cardiovascular health. Apart from diminishing cholesterol and triglyceride levels, kaempferol boosts cardiovascular performance, mitigates hypertrophic cardiomyopathy and has a potent inhibitory mechanism for platelet aggregation (Alrumaihi *et al*., 2024). Luteolin is another promising antioxidative molecule that has beneficial contributions to a range of diseases. Multiple research efforts disclosed that luteolin exerted a protective impact on cardiovascular health, particularly with regard to hypertension alongside associated ailments. Moreover, via intervening during the biosynthesis of cholesterol, luteolin has been apparent to substantially minimise cholesterol in model organisms (Queiroz *et al*., 2021).

Substantial empirical research is required in Ayurveda to ascertain the healthcare integrity and effectiveness of medications, with particular emphasis on quality objectives. Numerous research outcomes have indicated that the integration of conventional knowledge with qualitative attributes may render these formulations an invaluable resource within the healthcare system (Verma *et al*., 2024). Implementing HPTLC fingerprint features to establish pharmacopoeial benchmarks across a multitude of herbal formulations has become a crucial component of quality control. Assessing the phytochemical components present within formulations and determining their qualitative traits has become more straightforward through the implementation of such analytical techniques (Vyas *et al*., 2023). Moreover, no HPTLC strategy concerning the analysis of luteolin and kaempferol in Arjunarishta has previously been developed. Therefore, a strategy was implemented to analyse them using HPTLC.

Material and Methods

Materials

Terminalia arjuna bark, Madhuca indica flowers, and *Woodfordia fruticosa* flowers were gathered from varying sites in the Bhandara district of Maharashtra in India. The Botany Department of RTMNU, Nagpur, stated the veracity of the gathered specimens. The yeast culture was obtained in the laboratory by employing Madhuca indica flowers, whereas the standard strain of Saccharomyces cerevisiae SC1011 was received from the Kamalprakash Pharmacy College and Research Centre, Karanja, Maharashtra, India. Jaggery and fruits of Vitis vinifera were bought from the area's stores. All of the chemicals employed during the conduct of the investigation, ranging from solvents, had an adequate analytical level. As an investigation, four Arjunarishta formulations were examined, encompassing AM (marketed formulation), AI (produced through a non-conventional approach implementing isolated yeast inoculum within optimised circumstances), AS (produced through a non-conventional approach implementing the standard Saccharomyces cerevisiae yeast strain within optimised circumstances), and AT (formulated through the traditional mode).

Preparation of Arjunarishta Formulations

Terminalia arjuna powder (480 g) was allowed to soak in distilled water for an entire night, the powder was decocted by boiling it until roughly 25% of the water remained, and then jaggery (480 g) was incorporated. Finally, flowers of *Woodfordia fruticosa* and *Madhuca indica* (each 96 g) and fruits of *Vitis vinifera* (240 g) were introduced (Anonymous, 2008; Sahare & Khatri, 2024). Following appropriate sealing, the flask was laid out in a darkened location for the remainder of the task of fermentation. Such conventional formulation was assigned the designation ‘AT’. To generate unconventional formulations, a standard or isolated inoculum of *Saccharomyces cerevisiae* was employed in addition to particular fermentation settings such as an incubation temperature of 30.5°C, a jaggery concentration of 40% w/v, and an inoculum volume of 5.5% v/v. Statistical experimental design (Box-Behnken design) was adopted for optimising such circumstances. The identical procedure stated within the conventional mode was executed for generating a decoction. Jaggery was stirred into the decoction and autoclaved at 121°C for twenty minutes. The other ingredients were introduced a day later. Lastly, a 5.5% v/v yeast

inoculum, obtained from the flowers of *Madhuca indica* was introduced. The flask was labelled "AI" and stored in the incubator at 30.5°C after being properly sealed. *Saccharomyces cerevisiae* SC1011, a standard yeast strain, was used in place of the isolated yeast in the preparation of the additional unconventional formulation, which was given the designation "AS."

HPTLC Study

Preparation of Standard Solutions

To reach the intended ultimate concentration of 30 µg/mL, standard solutions of luteolin and kaempferol were created individually with methanol (Taco *et al*., 2024; Mali & Goyal, 2020).

Selection of Mobile Phase

The research reports of related investigations provided the foundation for the monitoring and evaluation of the numerous deployed mobile phases. Multiple solvent pairings were attempted to attain well-resolved bands, and ultimately the solvent system that emitted sharp and well-resolved bands was chosen as the mobile phase (Mali & Goyal, 2020; Hidayatullah, Yuwono & Primaharinastiti, 2022; Majumder & Saha, 2025).

Selection of Wavelength

Upon putting 10 µL of a mixed standard stock solution carrying 30 µg/mL of luteolin and kaempferol to the TLC plate, the chromatogram was let to develop. Employing reflectance mode, the two different bands were scanned to find the maximum wavelength.

Chromatographic Conditions

A Camag TLC device featuring a glass twin trough chamber, Camag Linomat 5 automatic TLC sample spotter, and Camag scanner 4 with incorporated visionCATS 4 software was implemented throughout the experiment. For installing 8 mm wide bands to aluminium plates that had already been pre-coated with Silica Gel 60 F254 (Merck), an automated TLC sampler was deployed. The spots stood apart by 1.14 cm and 2 cm from the plate's edges, including its base. In a Camag twin trough chamber that had been pre-saturated with twenty millilitres of mobile phase, the linearly ascending development proceeded. Formic acid, toluene, and ethyl acetate were incorporated within the proportions that follow the ratio 10.4:8.2:1.4 v/v/v for fifteen minutes under room temperature (25 ± 2°C) along with 30–40% relative humidity. The entirety of the chromatogram run was 7 cm. After development, TLC plates were dried and densitometrically scanned with vision CATS software and a Camag TLC scanner operating in the absorbance reflectance scan mode. A quantitative assessment of the plate in the absorption reflection mode at 365 nm was accomplished by employing tungsten and deuterium lamps.

Preparation of Calibration Curve

A mixed standard solution (luteolin and kaempferol, each 30 µg/mL) had been transferred into a TLC plate across various quantities (200–1000 ng/band). Furthermore, the plate had been developed and scrutinised. Calibrating the peak area in opposition to the appropriate deployment levels yielded calibration curves for both luteolin and kaempferol (Mali & Goyal, 2020; Patil *et al*., 2024).

Extraction of Phytoconstituents from Arjunarishta Formulations

Four Arjunarishta formulations (AI, AS, AT, and AM) were submitted to sonication, centrifugation, and supernatant layer concentration. After being filtered, the residue was adopted for analysis (Hlatshwayo *et al*., 2025).

Analysis of Luteolin and Kaempferol in Arjunarishta Formulations

Exactly 30 µL of individual standards, a mixture of standards, and each sample were introduced independently, developed, and a densitogram was established. The quantification was done utilising the peaks that aligned to luteolin and kaempferol.

Validation of developed HPTLC method

To ascertain the usefulness of the devised HPTLC method, the following parameters by alignment with ICH criteria were validated (Mali & Goyal, 2020; Patil *et al*., 2024; Borman & Elder, 2017).

i. **Linearity:** Using luteolin and kaempferol concentrations ranging from 200 to 1000 ng/band, a calibration curve was established to verify the linearity.

ii. **Sensitivity (LOD and LOQ):** It had been examined through the Sigma methodology. Following the introduction of a sample series for luteolin and kaempferol (200–1000 ng band-1), the plates were examined. Here, the detection, as well as quantification limits, had been set by adopting the signal-to-noise correlation methods.

iii. **Precision:** To acquire the task, three different strengths of luteolin and kaempferol solution (200, 300, and 400 ng band-1) were evaluated. These solutions were tracked in order to calculate an intra-day precision, as well as three days in succession, an inter-day precision with equivalent strengths was established.

iv. **Robustness and Ruggedness:** The detection wavelength (±2 nm) and saturation time (±5 min) were altered slightly, and the response results were achieved. The robustness approach was monitored with luteolin and kaempferol (300 ng band-1) while the ruggedness approach was attempted through two different investigators under comparable experimental and environmental conditions.

v. **Accuracy:** To establish the accuracy, the Addition approach was adopted. The 300 ng/band levels of experimental Arjunarishta samples were spiked at three distinct levels (80%, 100%, and 120% of the standards).

Results

Preparation of Arjunarishta Formulations

Certain fermentation parameters, such as fermentation temperature (30.5°C), substrate concentration (40%w/v), and yeast inoculum (5.5%v/v), were optimised by statistical experimental design (Box-Behnken design) and used throughout fermentation in unconventional formulations (AI and AS). The unconventional method revealed that the longest period needed to finish the task of fermentation was 12 days for AS and 15 days for AI. Conversely, the conventional formulation, AT, formed without any particular fermentation criteria and indicated that the fermentation process ceased after 24 days.

HPTLC Study

Selection of Mobile phase

To adequately separate the luteolin and kaempferol and cause well-resolved bands, various mobile phases were examined. In these circumstances, the mobile phase comprised Toluene, Ethyl Acetate, and Formic Acid with a proportion of 10.4:8.2:1.4 v/v/v which turned out to be reliable enough to yield discrete and noticeable bands of luteolin and kaempferol, sequentially with Rf values of 0.463 and 0.35.

Selection of Wavelength

The absorption overlaying spectrum of the luteolin and kaempferol standard solutions is illustrated in Figure 1. Since each compound demonstrated notable absorbance at 365 nm, this specific wavelength was assigned for quantifying the luteolin and kaempferol throughout the samples.

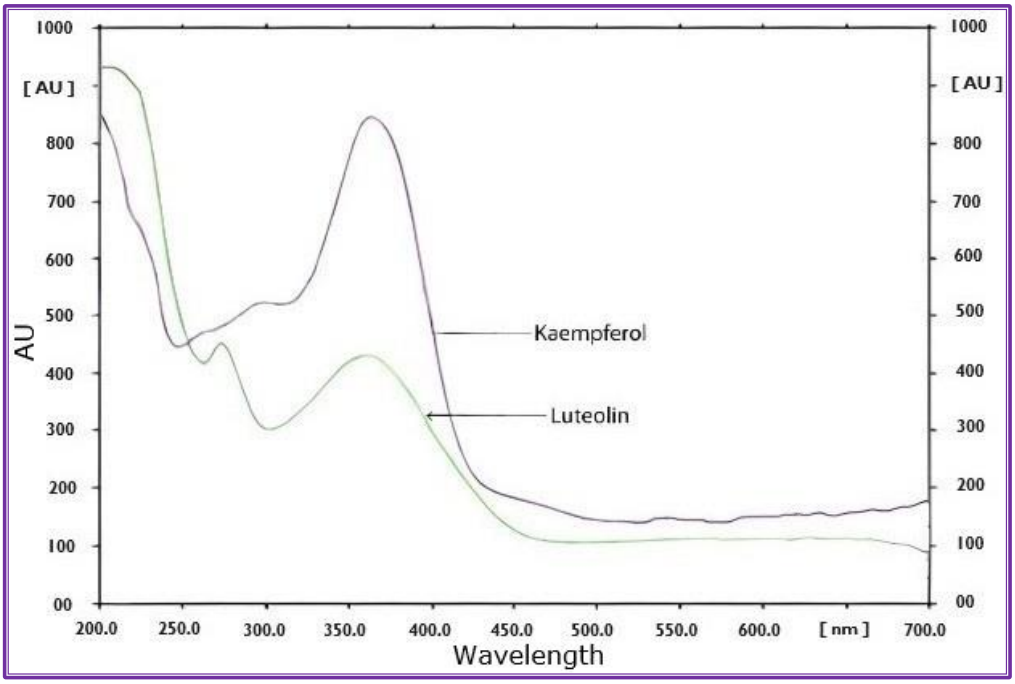

Figure 1: *Overlay Absorption Spectra of Kaempferol and Luteolin*

Analysis of various Arjunarishta Formulations

The differentiated bands displayed over a chromatographic plate as well as densitograms of standard substances and employed Arjunarishta specimens are illustrated in Figures 2 to 4. The experimental approach facilitates simultaneous enumeration of luteolin and kaempferol within the employed Arjunarishta specimens (AI, AS, AT, and AM) with appropriate resolution. The concentrations of luteolin and kaempferol within every specimen are disclosed in Table 1.

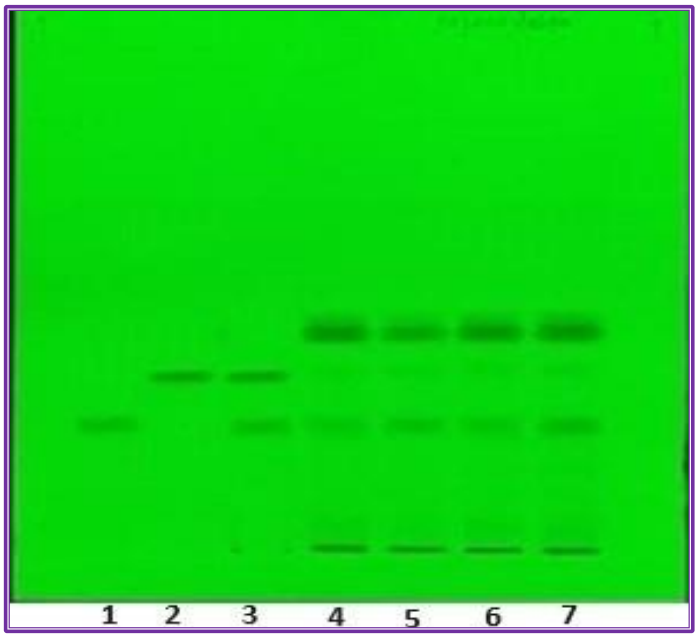

Figure 2: *Developed HPTLC plate for (From Left to right) (1) Kaempferol, (2) Luteolin, (3) Mixed standard solution, (4) AI, (5) AS, (6) AT and (7) AM formulations*

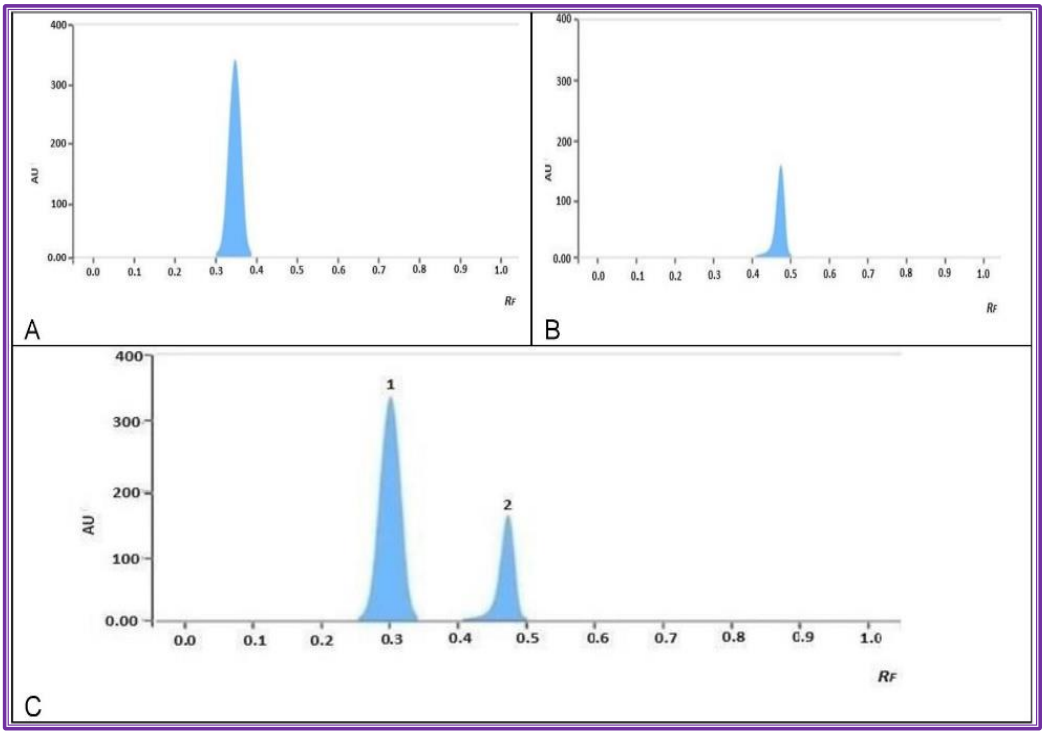

***Figure 3**: A. Densitogram of standard Kaempferol (RF=0.35); B. Densitogram of standard Luteolin (RF= 0.463); C. Densitogram of mixed standard solution showing (1) Kaempferol (RF= 0.305) and (2) Luteolin (RF= 0.463)*

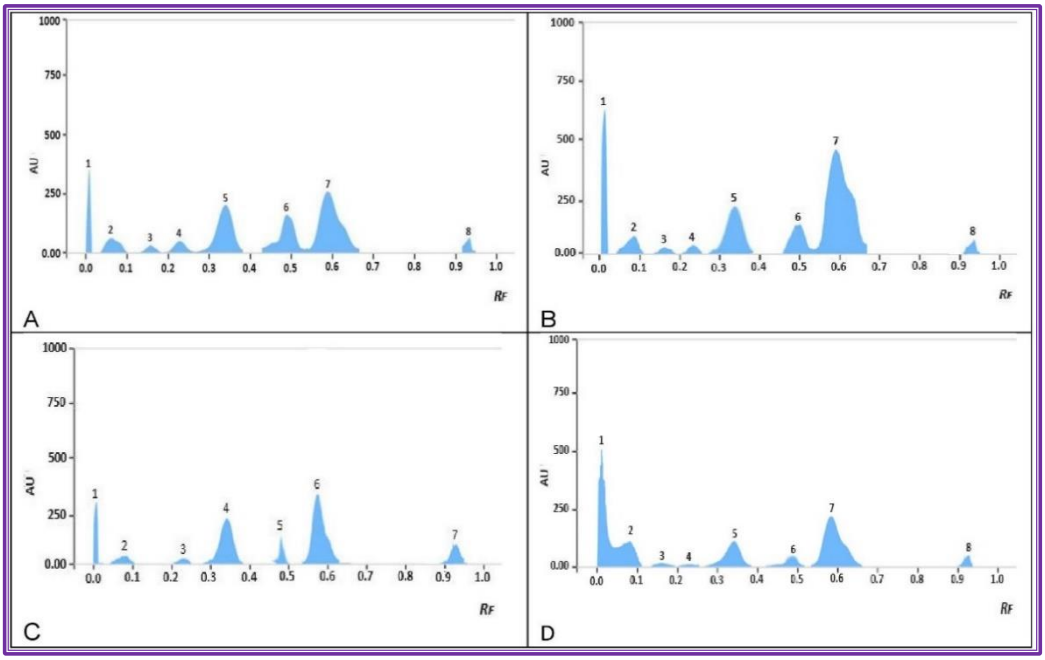

***Figure 4**: A. Densitogram of AI showing (5) Kaempferol (RF 0.342) and (6) Luteolin (RF 0.482); B. Densitogram of AS showing (5) Kaempferol (RF 0.343) and (6) Luteolin (RF 0.488); C. Densitogram of AT showing (4) Kaempferol (RF 0.344) and (5) Luteolin (RF 0.486); D. Densitogram of AM showing (5) Kaempferol (RF 0.341) and (6) Luteolin (RF 0.497)*

Table 1: *Content of Kaempferol and Luteolin in Various Arjunarishta Formulations*

Sample	Kaempferol Content (%W/V)	Luteolin Content (%W/V)
AI	0.355 ±0.689	0.285 ±1.04
AS	0.361 ±0.585	0.267 ±1.18
AT	0.342 ±0.91	0.135 ±1.30
AM	0.224 ±1.58	0.106 ±0.98

The values are expressed as mean ± SD through triplicate measurement.

Validation of Method

Linearity: Luteolin and kaempferol laid out substantial correlation values of 0.9979 and 0.9965, respectively, when the peak area of the resolved band was positioned against concentration (Figures 5). An adequate linear relationship over the concentration levels within the study has been explained by the regression stats, depicted in Table 2, establishing that the analysis satisfies the necessary criteria for investigation.

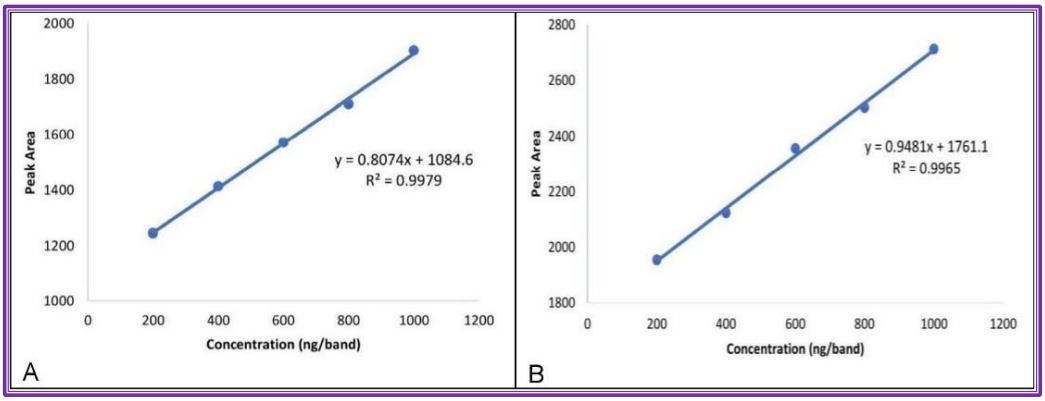

Figure 5: *Linearity of calibration curve for A. standard Luteolin and B. standard Kaempferol*

Table 2: *Linear Regression Data for Calibration Curves*

Parameter Studied	Results	
	Luteolin	Kaempferol
Linearity range	200-1000 ng	200-1000 ng
Regression equation	y = 0.8074x + 1084.6	y = 0.9481x + 1761.1
R2 ± %RSD	0.9979 ± 0.29	0.9965 ± 0.47
Slope ± %RSD	0.8074 ± 1.59	0.26

Sensitivity (LOD and LOQ): Kaempferol and luteolin were located to belong with detection limits of 1.48 and 0.68 ng spot-1, respectively, and quantification limits of 4.5 and 2.08 ng band-1, consequently. These findings imply that the protocol is appropriately sensitive.

Precision: Appraisals of luteolin as well as kaempferol solutions were executed by implementing a trio of levels (200, 300, and 400 ng band-1). The experiment addressed intra- and inter-day precision based on triplicate inspections for the mentioned compounds across the three distinguished suggested values. The values specified in Table 3 demonstrate the method's precise approach.

Table 3: *Precision Studies for Luteolin and Kaempferol*

Phytoconstituent	*Quantity (ng/band)*	*Concentration found (ng band-1)*	
		Intra-day	Inter-day
Luteolin	200	200.12 ± 0.15	198.54 ± 0.98
	300	296.12 ± 0.23	293.14 ± 1.14
	400	400.06 ± 0.67	388.25 ± 0.87
Kaempferol	200	200.05 ± 0.52	195.36 ± 1.56
	300	298.06 ± 1.35	294.31 ± 0.74
	400	399.26 ± 1.67	392.15 ± 0.26

The values are expressed as mean ± SD through triplicate measurement

Reproducibility (Robustness and Ruggedness): The percentage RSD readings remained under two percent, based on the robustness findings. As a consequence, the data imply that the recommended method is robust. The recommended approach proved to be reliable when the trial was conducted using different analysers measuring a concentration of 300 ng/band under equivalent operational and environmental conditions. The observations, particularly those presented in Table 4, indicated that the evaluation approach remained unaltered even with slight changes in the analytical conditions.

Table 4: Outcomes from the Ruggedness and Robustness Study

Reproducibility Condition	Parameter	Results	
		Luteolin (ng/band)	Kaempferol (ng/band)
Analyst-to-analyst variation	Analyst-1	300.23 ±1.06	300.22 ±1.02
	Analyst-2	300.10 ±0.987	295.97 ±1.13
	Analyst-3	300.10 ±1.02	300.21 ±0.85
	Mean	300.14 ±1.02	300.22 ±1.0
Wavelength (±2nm)	363 nm	298.78 ±1.56	294.91 ±1.28
	367 nm	300.14 ±1.37	300.23 ±1.36
Saturation time (±5 min)	15 min	300.09 ±1.26	300.08 ±1.09
	25 min	300.18 ±1.14	300.07 ±1.12

The values are expressed as mean ± SD through triplicate measurement

Accuracy (Percentage Recovery): Recovery assessments were followed through three distinct stages, i.e., 240, 300, and 360 ng band-1, sequentially representing 80%, 100%, and 120% of the recovery, to ascertain the accuracy of the approach. The closer an outcome gets to its correct values, the more accurate an approach to analysis. The recovery data (Table 5) hinted that the approach was appropriate within the allocated range.

Table 5: Recovery studies for Luteolin and Kaempferol

Samples	% Level	Initial amount of samples (ng/band)	Spiked amount of standards (ng/band)	Total expected amount (ng/band)	Amount recovered (ng/band)	% Recovery of Luteolin	% Recovery of Kaempferol
AI	80	300	240	540	539.6	99.92	98.68
	100	300	300	600	600.2	100.03	98.96
	120	300	360	660	659.1	99.86	99.78
					Mean	99.93	99.14
AS	80	300	240	540	540.8	100.14	99.72
	100	300	300	600	596.30	99.38	100.01
	120	300	360	660	657.2	99.57	99.86
					Mean	99.69	99.90
AT	80	300	240	540	536.13	99.28	99.49
	100	300	300	600	592.9	98.81	99.21
	120	300	360	660	660.9	100.15	100.03
					Mean	99.41	99.57
AM	80	300	240	540	532.3	98.57	98.34
	100	300	300	600	593.81	98.96	99.35
	120	300	360	660	644.72	97.68	99.59
					Mean	98.40	99.04

Discussion

The potency of herbal-based medicines can be affected by numerous factors, one of which is the lack of stringent standardisation. Therefore, standardising raw ingredients and manufactured medicines becomes a mandatory step to address quality-related factors (Das *et al*., 2019). Multiple analytical approaches are implemented to assess the quality criteria of herbal medicines. Deploying HPTLC

fingerprint profiling constitutes a crucial component in the surveillance of quality. As technology progresses rapidly, HPTLC is emerging as the method of choice for assessing the quality of herbal products. Despite its primary purpose being identification, the HPTLC method also facilitates comparison between standard and sample specimens, functioning as a versatile quality control tool. Peak variations and intensity ranges are generated through HPTLC fingerprinting, providing data that are both qualitative and quantitative, aligning with predetermined standards (Vyas *et al*., 2023).

The HPTLC method was deployed to analyse key phytochemicals in Arjunarishta, including gallic acid, ellagic acid, quercetin, rutin, and catechin (Sahare & Khatri, 2024; Thakur, Patil & Gawhankar, 2020). Kaempferol and luteolin are crucial phenolic components found in the bark of *Terminalia arjuna* (Verma & Jogdand, 2021). Multiple research reports have highlighted the preventive and antioxidative benefits of these compounds on cardiovascular health, particularly their ability to reduce cholesterol and lower blood pressure (Alrumaihi *et al*., 2024; Queiroz *et al*., 2021). The present research, representing the first examination of these compounds within the formulation, sought to evaluate the presence of luteolin and kaempferol in Arjunarishta using an HPTLC technique, taking into account their curative effects.

Through the evident separation and visualisation of luteolin and kaempferol, HPTLC facilitates the qualitative analysis. Retention factor values of the compounds that were separated are then compared with established standards for verifying the authenticity of these phytochemicals. This is a vital aspect for ensuring the quality and genuineness of the herbal composition. HPTLC quantifies the intensity of the separated spots that equate to luteolin and kaempferol to acquire the purpose of quantitative analysis. To ascertain the precise level of these components in Arjunarishta, this quantitative data is crucial. Precise measurement implies that the finished product complies with safety criteria and leads to the anticipated therapeutic benefits. Since HPTLC fingerprint analysis during the investigation revealed the existence of luteolin and kaempferol, which have already been scientifically researched for their antioxidative and cardioprotective efficacy, the current analysis affirms the curative qualities of Arjunarishta owing to the presence of these bioactive phyto-components.

The research being conducted adopted the Box–Behnken design to analyse the interactive effects of temperature, substrate concentration, and inoculum volume on the fermentation efficiency during the preparation of the Arjunarishta formulation. ANOVA results confirmed the model's significance ($p < 0.05$), with a high R^2 value, non-significant lack of fit, and close alignment between predicted and experimental values, indicating model fitness. The assessed optimal fermentation conditions were further verified through response surface graphs and regression equations, highlighting the crucial role of statistical optimisation in enhancing formulation quality and reproducibility.

The incorporation of isolated or standard *Saccharomyces cerevisiae* yeast inoculum under optimised fermenting conditions led to non-traditional Arjunarishta formulations that exhibited an accelerated rate of fermentation as well as enhanced concentrations of luteolin and kaempferol.

Established HPTLC analysis revealed that the developed Arjunarishta formulation contains a higher flavonoid content than the commercial version. The use of high-quality, fresh raw materials and optimal fermentation conditions may have contributed to this improvement by preserving and potentially enhancing bioactive components such as kaempferol and luteolin, thereby improving formulation quality and therapeutic potential.

The rigorous qualitative and quantitative analysis of kaempferol and luteolin via HPTLC not only confirms the presence and concentration of these compounds but also ensures the therapeutic efficacy and safety of Arjunarishta. Such scientific validation is crucial for establishing Arjunarishta as a reliable formulation for managing circulatory system disorders through a qualitatively assured, effective approach.

Conclusion

It turns out that luteolin and kaempferol can be estimated simultaneously in Arjunarishta using a reliable and effective method established through the HPTLC analysis conducted during the execution of this

study. The presence of both bioactive components was evidenced by appropriate resolution in the chromatographic separation, highlighting the qualitative aspects of Arjunarishta. In conclusion, the implementation of this approach in such Ayurvedic formulations is supported by its validation, indicating that the method is compatible with Arjunarishta's quality evaluation. Future studies will focus on adopting multi-wavelength approaches to detect additional phytoconstituents, thereby addressing the limitations of single-wavelength detection. Furthermore, stability studies are proposed to evaluate degradation behaviour and ensure the long-term efficacy of the formulation.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgement

The authors would like to extend their gratefulness to the Faculty of Pharmacy, Sardar Patel University, India for the accomplishment of this research work.

References

Alrumaihi, F., Almatroodi, S. A., Alharbi, H. O. A., Alwanian, W. M., Alharbi, F. A., Almatroudi, A., & Rahmani, A. H. (2024). Pharmacological potential of kaempferol, a flavonoid in the management of pathogenesis via modulation of inflammation and other biological activities. *Molecules, 29*(9), 2007. https://doi.org/10.3390/molecules29092007

Anonymous. (2008). The Ayurvedic Pharmacopoeia of India, Part II (Formulations), Volume II (p. 47). Government of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy. Available at: https://naturalingredient.org/wp/wp-content/uploads/API-II-Vol-2.pdf

Borman, P., & Elder, D. (2017). Q2 (R1) validation of analytical procedures: text and methodology. *ICH quality guidelines: an implementation guide*, 127-166. https://doi.org/10.1002/9781118971147.ch5

Das, C., Ghosh, G., Bose, A., & Das, D. (2019). Analytical methods for standardization of Ayurvedic Asavas and Aristas; A review. *Indian Journal of Pharmaceutical Sciences*, *81*(3), 396-405. https://doi.org/10.36468/pharmaceutical-sciences.523

Hidayatullah, M., Yuwono, M. & Primaharinastiti, R. (2022). Optimization Method and Stability Test forDetermination of Luteolin, Quercetin, Apigenin, Sinensetin Levels in Herbal Medicines Using TLC-Densitometry. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia, 9*(3), 235-241. https://doi.org/10.20473/jfiki.v9i32022.235-241

Hlatshwayo, S., Thembane, N., Krishna, S. B. N., Gqaleni, N., & Ngcobo, M. (2025). Extraction and processing of bioactive phytoconstituents from widely used South African medicinal plants for the preparation of effective traditional herbal medicine products: a narrative review. *Plants*, *14*(2), 206. https://doi.org/10.3390/plants14020206

Majumder, S. & Saha, P. (2025). Quantitative estimation of quercetin from Phoenix sylvestris using validated HPTLC method. *International Journal of Pharmaceutical Sciences and Research, 16*(1), 162-66. https://doi.org/10.13040/IJPSR.0975-8232.16(1).162-66.

Mali, P. Y., & Goyal, S. (2020). HPTLC densitometric quantification of kaempferol from leaves of Euphorbia neriifolia. *Indian Journal of Pharmaceutical Education and Research*, *54*, s586-s592. https://doi.org/10.5530/ijper.54.3s.158

Patil, S., Khushwah, P., Gudasi, S., Patil, M., Kunchanur, M., & Koli, R. (2024). Simultaneous Determination of Gallic Acid, Berberine and Trigonelline in Polyherbal Churna by HPTLC Method. *Journal of Young Pharmacists*, *16*(2), 261-268. https://doi.org/10.5530/jyp.2024.16.34

Queiroz, M., Leandro, A., Azul, L., Figueirinha, A., Seiça, R., & Sena, C. M. (2021). Luteolin improves perivascular adipose tissue profile and vascular dysfunction in Goto-Kakizaki rats. *International Journal of Molecular Sciences*, *22*(24), 13671. https://doi.org/10.3390/ijms222413671

Sahare, A. Y. & Khatri R. (2024). Review of arjuna Rishta: bridging ancient ayurvedic tradition with contemporary scientific validation. *International Journal of Creative Research Thoughts, 12*(11), c586- c591. Available at: https://www.ijcrt.org/papers/IJCRT2411289.pdf

Singh, A., Tiwari, R. C., Dikshit, M., Sharma, V. B., Mittal B. & Kumar N. (2025). Herbal science: exploring the phytochemistry and pharmacognosy of medicinal plants. *International Ayurvedic Medical Journal*, Online, 72-78. https://doi.org/10.46607/iamj1113012025

Taco, V., Palmieri, C., Borja, D., Villacrés, E., Duez, P., & Nachtergael, A. (2024). Qualitative Analysis by High-Performance Thin-Layer Chromatography–Bioautography of Ecuadorian Chenopodium quinoa Willd. Leaves:

Influence of Variety, Phenological Stage, and Place of Cultivation on Free Radical Scavenging and α-Amylase Activity. *Nutraceuticals*, *5*(1), 1. https://doi.org/10.3390/nutraceuticals5010001

Tambe, G. A., Singh, R., Yadav, P., & Prajapati, P. K. (2023). The status of fermented preparations in clinical practice: A charaka samhita review. *Journal of Indian System of Medicine*, *11*(3), 176-183. https://doi.org/10.4103/JISM.JISM_2_23

Thakur, K. S., Patil, P., & Gawhankar, M. (2020). Standardization of Ayurvedic Formulation "Arjunarishta" In Terms of Physicochemical, Spectroscopy and Chromatographic Techniques.I*nternational Journal of Pharmaceutical Sciences and Research 11*(12), 6237-6242. http://dx.doi.org/10.13040/IJPSR.0975-8232.11(12).6237-42

Verma, S. K., Pandey, M., Sharma, A., & Singh, D. (2024). Exploring Ayurveda: principles and their application in modern medicine. *Bulletin of the National Research Centre*, *48,* 77. https://doi.org/10.1186/s42269-024-01231-0

Verma, V. & Jogdand, S. (2021). Medicinal Properties of *Terminalia arjuna*: A Review. *Journal of Pharmaceutical Research International*, 33(62A), 593-604. http://dx.doi.org/10.9734/jpri/2021/v33i62A35893

Vyas, A., Jain, V., Sahu, U., Kumar, N., & Joshi, N. (2023). HPTLC method development of herbal drugs and its validation: An overview. *Research Journal of Pharmacy and Technology*, *16*(8), 3964-3976. https://doi.org/10.52711/0974-360X.2023.00652