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Validated HPTLC method for rapid quantification of galangin in *Alpinia officinarum*: A cost-effective approach

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Abstract
Alpinia officinarum (Hance), a member of the Zingiberaceae family, is a significant medicinal rhizome that is used in Indian traditional medicine to treat a variety of illnesses. In the herbal drug industry, adulteration of closely related plant species within the Zingiberaceae family has emerged as a significant problem. The current work aims to create an analytical method for determining galangin, a main ingredient of *A. officinarum*, that is easy to use, quick, selective, and economical. For the quantitative and qualitative assessment of galangin in *A. officinarum*, the High Performance Thin Layer Chromatography (HPTLC) densitometric method was chosen. The findings of current study demonstrated that the techniques employed were easy, precise, specific, consistent, sensitive and accurate. As such, they may be applied to regular quality control of raw materials and formulations including galangin as well as the quantification of galangin.

Keywords: *Alpinia officinarum*, galangin, HPTLC, cost effective approach

Introduction
Traditional plant-based remedies have been employed for centuries to address diverse ailments, with a notable surge in utilization over the past decade. The World Health Organization (WHO) reports that 11% of important drugs are produced from plants, and that 80% of the world's population currently uses herbal remedies for their primary medical requirements (Haq, 2004) [7]. In India, Ayurveda and Unani medicine have been of prime importance and use specific phytochemicals as remedy for curing various diseases (Hamadani *et al.*, 2018) [6]. The Zingiberaceae family of plants is incredibly abundant in medicinal herbs that have been used for centuries as spices and to treat a wide range of ailments. Most of these species are found in India as well as extensively throughout the tropics of world (Basak *et al.*, 2010) [3]. Alpinia, a prominent genus in the Zingiberaceae family, has been utilized for a long time for both therapeutic and non-therapeutic purposes. Among them, the noteworthy species are *Alpinia oxyphylla*, *Alpinia zerumbet*, *Alpinia galanga* (larger galangal), and *Alpinia officinarum* (lesser galangal), all of which have important pharmacological roles.

Alpinia officinarum, a perennial herb and medicinal food plant, primarily grown in Southeast Asia, but it originated in China. The plant was traditionally used as spices and flavouring agent. The dark reddish-brown rhizomes known as galangal were traditionally used to cure whooping cough and rheumatism. (Srividya *et al.*, 2010) [12]. The plant has been reported to possess potent anti-inflammatory, antibacterial, antifungal, antiviral, antioxidant, diuretic and anticancer properties which are attributed mainly due to the array of phytochemicals associated with the herb. The plant is used to cure digestive disorders, inflammation, common colds and other conditions either alone or in combination with other herbs (Abass *et al.*, 2018; Abubakar *et al.*, 2018 and Bitari *et al.*, 2023) [1, 2, 4].

Various phytochemical constituents such as quercetin, alpinol, 1,8-cineole, methyl cinnamate, α-cadinene, galangin, 3-O-methyl galangin, kaempferide, alpinin, galangol, and certain diarylheptanoids were found to be present in *A. officinarum* (Shin *et al.*, 2003) [11]. Galangin, a major flavonol, appeared to be the primary constituent in the rhizomes of *A. officinarum*, exhibiting multiple pharmacological properties (Zhai *et al.*, 2014; Thapa *et al.*, 2023) [16, 13].

The herbal drug industry faces a significant challenge of substitution with closely related plant species within the Zingiberaceae family, owing to their similar physical characteristics. Hence, it is imperative to establish an analytical method for precise herbal discrimination to ensure

accurate identity and authenticity (Pauzi, 2022) [8]. In the current study, a simple, sensitive and accurate HPTLC method was developed to identify and evaluate galangin in rhizomes of *Alpinia officinarum* cultivated at herbal garden of Ethno Veterinary Herbal Product Research and Development Centre, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur District, Tamil Nadu.

2. Materials and Methods

2.1 Collection of plant materials

Fresh rhizomes of *Alpinia officinarum* was collected from Herbal Garden, Ethno Veterinary Herbal Product Research and Development Centre, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur District, Tamil Nadu. The Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai-106, where the voucher specimens have been deposited in the herbarium, verified the authenticity of the plants. The rhizomes were manually washed, shade-dried (25±3 °C) and powdered using pulveriser (40 mesh). The powdered herbal powders were kept for later studies in an airtight container.

2.2 Preparation of plant extract

A Soxhlet apparatus was used to extract 100g of the powdered rhizome sample of *A. officinarum* for 72 hours using 500 ml of ethanol (Zhao *et al.*, 2019) [17]. The extracted solution was then evaporated by using Rotary Evaporator (Buchi Rotavapor R-300) to remove the solvent and stored at 4 °C until use.

2.3 Estimation of yield percentage

The extraction yield percentage of the plants used in the present study was calculated by using the following formula.

$$\text{Percentage of extraction} = \frac{\text{Weight of the extract (g)}}{\text{Weight of the powdered plant material (g)}} \times 100$$

2.4 Qualitative Phytochemical Screening

A preliminary phytochemical screening was performed on freshly prepared plant extracts to determine the presence of diverse phytochemicals (Trease and Evans, 1989, Sahu *et al.*, 2014) [14, 10].

2.5 Analytical studies

2.5.1 HPTLC protocol for the galangin quantification from *Alpinia officinarum*

2.5.1.1 Apparatus

Thin Layer Chromatography (TLC) plates precoated with silica gel 60 F254 (Merck, Darmstadt, Germany) were used for the chromatographic process. An automatic sample applicator, Linomat 5 (Camag, Muttenz, Switzerland), was used as the spotting device, along with a 100 µL Hamilton syringe (Hamilton Bonaduz, Switzerland). A banding application was made, with a distance of 8.0 mm placed on the Y axis of plate and a length of 8.00 mm maintained. In a twin-trough vertical development chamber (20 x 10), the plates were developed after being saturated with the mobile phase for 20 minutes (Camag, Switzerland). After the development process, the plate was scanned using vision CATS software (version 2.5.18262.1) (Camag, Switzerland) at a wavelength of 245 nm, at a scanning speed of 20 mm/s, and with a slit dimension of 6.0 mm x 0.45 mm.

2.5.1.2 Preparation of standard solution of galangin

Standard Galangin (10 mg) was dissolved in 10 mL of methanol to prepare a stock solution. A working standard solution (100 µg/mL) was made from this stock solution and put into 10 mL volumetric flasks, with the volume being adjusted using methanol.

2.5.1.3 Preparation of sample solution

The 10 mg dried rhizome extract of *Alpinia officinarum* was transferred to a 10 mL volumetric flask, and methanol was added to get the volume up to 10 mL to achieve the required final concentration of 1 mg/mL.

2.5.1.4 Calibration curve for galangin

Galangin standard solution of 100 ug/ml concentration was applied at eight different volumes (1, 2, 3, 4, 5, 6, 7 and 8 µL in triplicates) and herbal samples (1 mg/ml) were run in duplicates at seven different concentrations (1, 2, 3, 4, 5, 6 and 7). The plate was developed at 25±2 °C with 40% relative humidity up to 8 cm in a solvent system containing hexane: ethylacetate: acetic acid (7.5:2:0.5 v/v). After development, the plate was air-dried and scanned at 254 nm wavelength. The peak area was recorded. Calibration curve was prepared by plotting Peak area vs. Concentration.

2.5.1.5 Validation of the method

The International Conference on Harmonisation (ICH) Harmonised Tripartite guidelines (ICHHT, Q2 (R1) 2005) were followed to validate the proposed method.

2.5.1.5.1 Precision

The instrumental precision was tested by scanning (n=6) the identical galangin spot (300 ng/spot), and the results (n=6) were presented as a percentage relative standard deviation (% RSD). The variability of method was analyzed by examining aliquots of the galangin standard solution (200, 300, and 400 ng/spot) both during the same day (intraday precision) and between different days (interday precision). The results were expressed as a % RSD.

2.5.1.5.2 Repeatability

After application of 300 ng/spot of galangin standard solution on the TLC plate (n = 6), the repeatability of the method was evaluated and analyzed them as described in the preparation of calibration plot; the result was expressed as % RSD.

2.5.1.5.3 Robustness

The robustness of the proposed HPTLC densitometric method was assessed to evaluate the impact of small deliberate changes to the chromatographic conditions, such as the volume, composition, and duration of the mobile phase saturation as well as the activation of HPTLC plates during the galangin determination. Chromatograms were performed using mobile phases hexane-ethyl acetate-acetic acid at different composition *viz*. 7.0:1.5:0.5 v/v, 7.5:2.0:0.5 v/v and 8.0:1.0:1.0 v/v. The robustness of method has been evaluated at the 420 ng/spot concentration level for galangin.

2.5.1.5.4 Specificity

The standard and samples were analyzed to determine the specificity of method. By comparing their Rf values and spectra with accepted standards, the bands in the chromatogram that were obtained from the samples that

corresponded to galangin were confirmed. By comparing the standard and sample spectra obtained at the peak start [S], peak apex [M], and peak end [E] of the bands, the peak purity was determined.

2.5.1.5.5 Accuracy
Accuracy of the method was evaluated employing recovery studies conducted at three different levels: 80%, 100%, and 120%. Both the recovery percentage and the mean recovery percentage were calculated.

2.5.1.5.6 Limit of detection and Limit of quantification
The lowest concentrations of galangin that can be detected are represented by the LOD, whereas the lowest concentrations that can be estimated with an acceptable level of precision and accuracy are represented by the LOQ. A determination of LOD and LOQ was made using the signal-to-noise (S/N) ratio. The methanol was used as a blank, and the known concentrations of the galangin standard solution were applied until the average responses for the six repeat determinations were roughly 3 to 10 times the standard deviation of the responses.

3. Results and Discussion
3.1 Extraction yield percentage and phytochemical screening
The extraction yield percentage of *A. officinarum* ethanolic extract in the present study is 8% and the results of phytochemical screening is shown in Table 1.

Table 1: Phytochemical analysis of *Alpinia officinarum*

S. No.	Constituents	Result
1.	Carbohydrates	Positive
2.	Saponins	Negative
3.	Alkaloids	Positive
4.	Phenols	Positive
5.	Tannins	Positive
6.	Terpenoids	Positive
7.	Flavonoids	Positive
8.	Proteins	Negative
9.	Glycosides	Negative
10.	Cardiac glycosides	Negative

3.2 HPTLC studies of galangin for *Alpinia officinarum*
One of the most advanced instrumental methods for the qualitative and quantitative evaluation of herbal drugs is High Performance Thin Layer Chromatography (HPTLC) (Vidhyatai *et al*., 2022) [15]. It is one of the most often used methods in phytochemical analysis, because of its multiple benefits, including the ability to portray the data as an image, ease of use, minimal sample cleanup requirements, high sample capacity, parallel analysis of samples, potential for multiple detection, and affordability. Compared to HPLC, liquid chromatography, and electro-spray mass spectrometry, HPTLC requires significantly less time for sample analysis (Reich and Schibli, 2008) [9].

Therefore, the HPTLC densitometric method was chosen for the quantitative and qualitative assessment of galangin in *Alpinia officinarum*. With a retention factor (Rf) of 0.40±0.2, the hexane-ethylacetate-acetic acid (7.5:2:0.5 v/v) solvent system produced the greatest resolution of galangin among the many mobile phases tested. Fig 3.1 shows developed chromatographic plate of galangin.

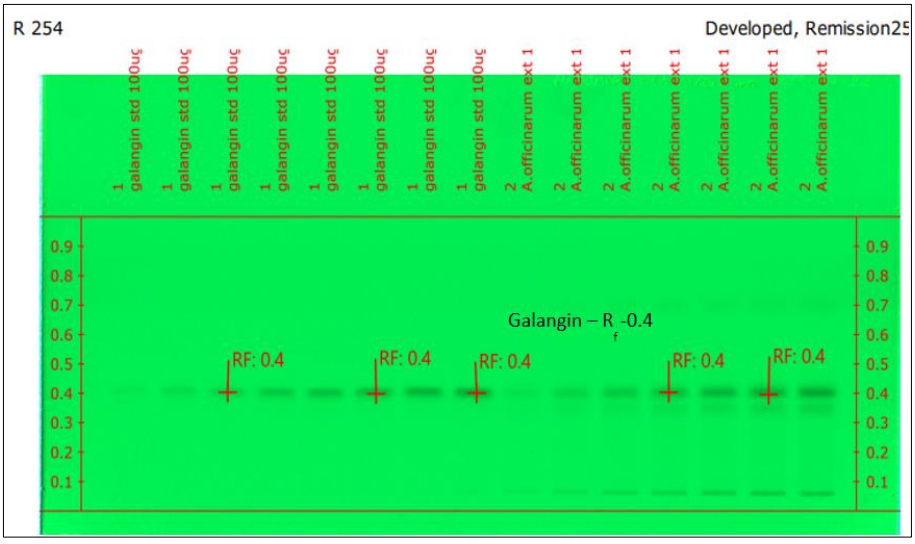

Fig 1: Developed chromatographic plate of galangin

3.3 Linearity of galangin
Linear regression demonstrated a strong correlation (r2) between the concentration of standard solutions and the peak response within the concentration range of 100 to 800 ng/spot with a correlation coefficient (r^2) of 0.999. Fig.3.3a shows linearity and Fig.3.3b shows densitogram of galangin.

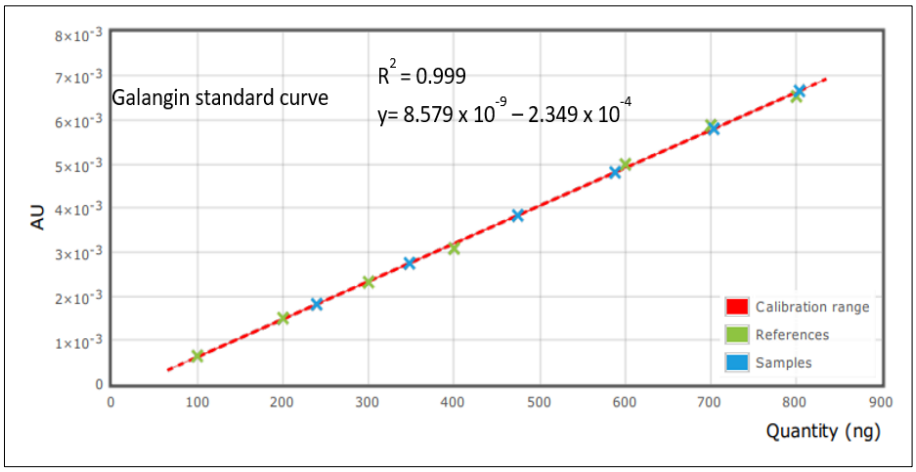

Fig 2: Linearity of galangin

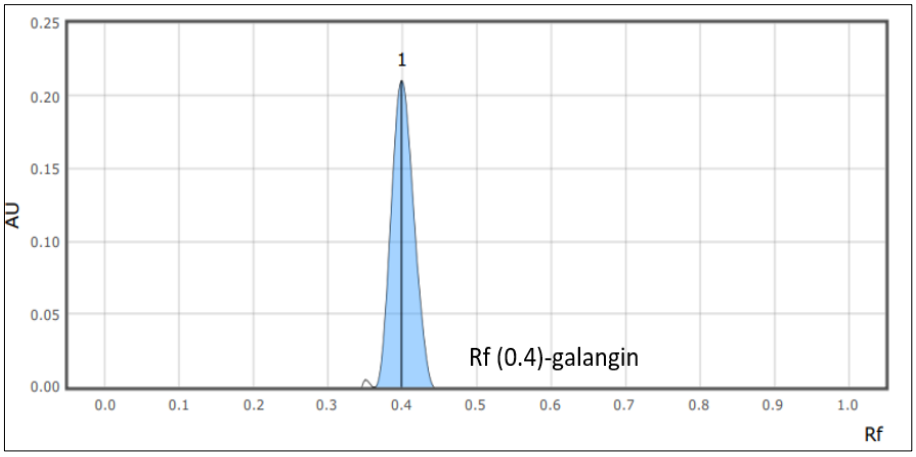

Fig 3: Densitogram of galangin

3.4 Limit of Detection (LOD) and Limit of Quantification (LOQ) for galangin

Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated to determine sensitivity as 3.3 σ/s and 10 σ/s, respectively, where S stands for the slope of the linearity plot and σ for the standard deviation of the response (y-intercept). The signal-to-noise ratios of 3.3 and 10 were used to determine the LOD and LOQ. For galangin, the obtained LOD and LOQ were 31.47 and 95.36 ng/spot, respectively. This suggested that the novel technique had good sensitivity to quantify galangin.

3.5 Precision and Repeatability

The robustness of the method is demonstrated by the precision and reproducibility at three different concentration levels. The intraday and inter day precision results are presented in Table. 3.5.

Table 2: Intraday and inter day precision of galangin

Standard drug	Nominal concentration	Concentration obtained		% RSD	
		Intra day	Inter day	Intra day	Inter day
Galangin	200	200.60	199.85	0.515	0.283
	300	298.84	299.4	0.455	0.374
	400	399.82	400.1	0.202	0.312

3.6 Robustness

The percentage Relative Standard Deviations (% RSD) was found to be less than 2% after the standard deviation of peak areas was calculated for each condition. These low values of % RSD was indicative of the robustness of the method and the results are presented in Table 3.6.

Table 3: Robustness of galangin

Concentration (ng/spot)	Mobile phase composition (hexane-ethylacetate-acetic acid)		Results (n=6)		
	Original	Used	Concentration ± SD	% RSD	R_f
420	7.5:2.0:0.5	7.0:1.5:0.5	419.86±0.82	0.197	0.38
		7.5:2.0:0.5	420.01±0.04	0.01	0.40
		8.0:1.0:1.0	419.07±0.54	0.129	0.39

3.7 Specificity

The visible spectra obtained at the peak start (S), peak apex (M) and peak end (E) of the peaks obtained by band scanning were compared to determine the peak purity for galangin. The acquired values were r (M, E) = 0.999 and r (S, M) = 0.999. The data on peak purity indicated that the peak observed for galangin was pure. Fig 3.7 shows the peak purity of galangin in standard and sample.

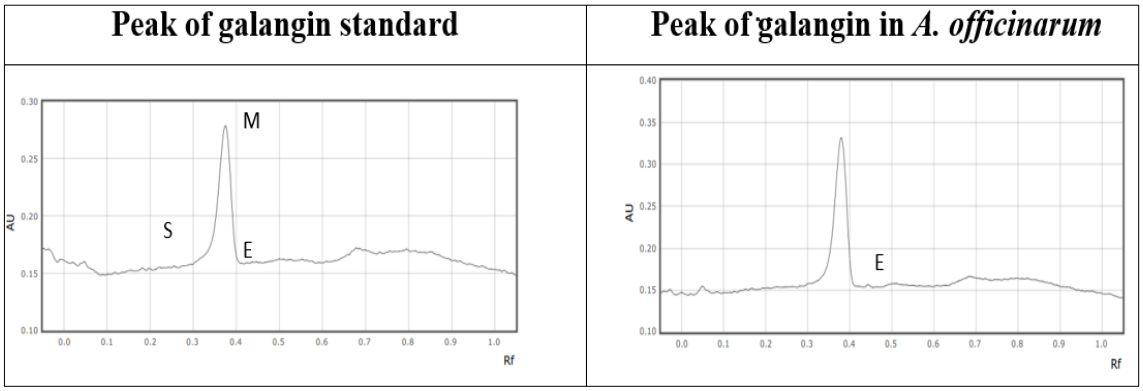

Fig 4: Peak purity of galangin in standard and sample

3.8 Accuracy

The accuracy of the method was evaluated by the recovery study and expressed as percentage recovery. The percentage recovery and average percentage recovery were calculated. The percentage of recovered galangin is determined to be between 99.89 and 100.01%, with RSD values between 0.055 and 0.114, following the addition of standard galangin to the same amount of the sample solution at three different concentration levels. The average percent recovery is found to be with an average of 99.95%. These results indicated the accuracy of the method and are presented in Table 3.8.

Table 4: Accuracy of galangin

Concentration taken	Concentration added	Concentration found	% Recovery	% RSD
300	240	540.05	100.01	0.055
300	300	599.80	99.96	0.029
300	360	659.28	99.89	0.114

3.9 Method validation for galangin

Table 5: HPTLC method Validation parameters for the quantitation of galangin

Parameters	Results
Linearity range	100 to 800 ng/spot
Correlation Coefficient	0.999
Limit of detection	31.468 ng/spot
Limit of quantification	95.36 ng/spot
Specificity	Specific
Robustness	Robust

3.10 Quantification of galangin in *Alpinia officinarum*

Calibration curve was generated using seven reference samples and seven lesser galangal samples for quantification purposes. The correlation coefficient was 99.935%, while the coefficient variation (CV) was 2.11%. The calibration curve indicated that galangin content in the sample is 165.5 µg/mg.

4. Conclusion

This study outlines an HPTLC technique for both qualitative and quantitative assessment of galangin. The proposed method demonstrates satisfactory reproducibility, repeatability and accuracy, as indicated by low standard deviation and percent relative standard deviation values. The approach is deemed simple, precise, specific, reproducible, sensitive and accurate, making it suitable for quantifying galangin and routine quality control of raw materials and formulations containing this compound.

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