

Comparative HPTLC-Based Quantitative Estimation of Quercetin in Selected Traditional Medicinal Plants of the Families Zingiberaceae, Malvaceae, and Ebenaceae

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ABSTRACT

Differences in the phytochemical profiles of herbal raw materials highlight the need for reliable marker-based standardization to maintain quality, safety, and therapeutic uniformity. Quercetin, a biologically active flavonoid glycoside, is commonly employed as a marker compound in the quality assessment of flavonoid-rich medicinal plants. This study was undertaken to develop a high-performance thin-layer chromatographic (HPTLC) technique for the quantitative estimation of quercetin in selected medicinal plant extracts commonly used in traditional medicine. Methanolic extracts of *Curcuma domestica*, *Alpinia galanga*, *Elettaria cardamomum*, *Gossypium arboreum*, and *Diospyros ebenum* were subjected to HPTLC analysis. Chromatographic separation was carried out on silica gel 60 F₂₅₄ plates using a mobile phase comprising toluene, ethyl acetate, formic acid, and methanol in the ratio of 3:6:1.6:0.4 (v/v/v/v). Densitometric evaluation was performed at 254 nm. Quercetin showed good resolution with R_f values between 0.82 and 0.89. Quantitative estimation indicated quercetin contents of 0.146% in *Curcuma domestica*, 0.310% in *Alpinia galanga*, 0.052% in *Elettaria cardamomum*, 0.131% in *Gossypium arboreum*, and 0.115% in *Diospyros ebenum*. The HPTLC fingerprint profiles exhibited satisfactory resolution and reproducibility. The developed method is straightforward, rapid, and dependable for quercetin quantification in medicinal plant extracts, supporting its applicability in quality control and standardization of flavonoid-rich herbal materials and traditional polyherbal formulations.

Keywords *Curcuma domestica*, *Elettaria cardamomum*, HPTLC, quercetin.

INTRODUCTION

Herbal medicines form the backbone of traditional healthcare systems worldwide and continue to gain global acceptance due to their therapeutic potential and natural origin. However, the quality, safety, and efficacy of herbal raw materials are often compromised by significant variability in their phytochemical composition. Factors such as geographical source, climatic conditions, genetic variation, harvesting season, processing methods, and storage conditions contribute to inconsistencies in the chemical profile of medicinal plants, leading to variations in therapeutic outcomes [1,2]. Therefore, scientific standardization and quality control of herbal drugs are essential to ensure their reproducibility and

clinical reliability. Marker-based standardization is widely recognized as an effective approach for quality assessment of herbal materials. This strategy involves the identification and quantification of specific bioactive or characteristic compounds that serve as indicators of quality and authenticity [3]. Among the diverse classes of phytoconstituents, flavonoids are of particular importance due to their widespread distribution in medicinal plants and broad spectrum of biological activities, including antioxidant, anti-inflammatory, antimicrobial, cardioprotective, and anticancer effects [4,5]. Quercetin, a naturally occurring flavonol glycoside, is one of the most extensively studied flavonoids and is commonly employed as a phytochemical marker in flavonoid-rich medicinal plants. It has been reported to exhibit

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strong free radical scavenging activity, inhibition of inflammatory mediators, modulation of cellular signaling pathways, and protection against oxidative stress-related disorders [6–8]. Owing to its pharmacological relevance, chemical stability, and abundance in numerous medicinal plants, quercetin is considered a suitable marker compound for the standardization and quality control of herbal drugs and formulations [9]. High-performance thin-layer chromatography (HPTLC) has emerged as a powerful and reliable analytical technique for the evaluation of herbal medicines. It offers several advantages over conventional chromatographic methods, including simplicity, low operational cost, minimal sample preparation, high sample throughput, and the ability to generate characteristic fingerprint profiles [10,11]. HPTLC is particularly well suited for herbal analysis as it allows simultaneous qualitative and quantitative estimation of marker compounds in complex plant matrices. Consequently, regulatory bodies such as the World Health Organization (WHO) and various pharmacopoeias recommend HPTLC for herbal drug standardization and quality control [2,12]. Several medicinal plants used in traditional systems of medicine, including *Curcuma domestica* (Zingiberaceae), *Alpinia galanga* (Zingiberaceae), *Elettaria cardamomum* (Zingiberaceae), *Gossypium arboreum* (Malvaceae), and *Diospyros ebenum* (Ebenaceae), are known for their therapeutic properties and rich flavonoid content. These plants are traditionally employed for their antioxidant, anti-inflammatory, digestive, antimicrobial, and wound-healing activities. [13–17]. Despite their extensive medicinal use, systematic quantitative estimation of quercetin in these plant species using HPTLC methods remains limited.

The present study was undertaken to develop a simple HPTLC method for the quantitative estimation of quercetin in selected medicinal plant extracts. The study further aims to establish HPTLC fingerprint profiles to support the use of quercetin as a phytochemical marker for quality control and standardization of flavonoid-rich herbal raw materials and traditional polyherbal formulations.

MATERIALS AND METHODS

Collection of Raw Materials:

The raw materials were obtained as gift samples from a traditional Siddha medical practitioner, Mr. Cinnathambi, Boomidi, Dharmapuri District. The identity of the collected materials was authenticated by Dr. S. Mutheeswaran, Scientist, Centre for Biodiversity and Biotechnology, Xavier Research Foundation, St. Xavier's College, Tirunelveli, Tamil Nadu, India.

Instruments:

The herbal raw materials were weighed in order to prepare extracts using a CAMAG HPTLC system that included a Linomat-V applicator, CAMAG TLC Scanner-3, and a Shimadzu model single pan balance.

Preparation of Standards and Extracts:

One gram of each dried and powdered herbal raw material- *Curcuma domestica*, *Alpinia galanga*, *Elettaria cardamomum*, *Gossypium arboreum*, and *Diospyros ebenum* —was extracted with 10 mL of methanol by sonication. The extracts were filtered, and the resulting filtrates were used for HPTLC analysis. Standard marker solutions were prepared in methanol at a concentration of 1 mg/mL.

Application of Sample:

Using a Hamilton 100 µL syringe and a Camag Linomat V applicator, the herbal formulation solutions were spotted as 4 mm wide bands on precoated plate 60 F254 (10 cm × 10 cm with 0.2 mm thickness, E. Merck). The dimensions of the slit were maintained at 6 × 0.45 mm. Five microliters of standard solutions and eight microliters of each herbal raw material extract were added to the plate. There was an 80 mm migratory distance. An air dryer was used to dry the TLC plates. Wincat software was used to control the Camag TLC Scanner-3 at 254 and 366 nm for densitometric scanning.

Development:

Sample and standard solutions were applied as 6 mm wide bands on precoated silica gel 60 F254 aluminium plates (10 cm × 10 cm, 0.2 mm thickness; E. Merck) using a Hamilton 100 µL syringe with a CAMAG Linomat-V applicator. The slit dimensions were maintained at 6.0 × 0.45 mm. Five microliters of



standard solution and 8 μ L (or 5 μ L where specified) of sample extracts were applied. The migration distance was maintained at 80 mm. Plates were air-dried prior to development. Chromatographic separation was carried out in a CAMAG twin -trough glass chamber (10 $\times$ 10 cm) pre-saturated for 10 min at $25 \pm 1^\circ\text{C}$ and 40% relative humidity. The mobile phase comprised toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4, v/v/v/v). The Plates were developed to a migration distance of 8 cm from the origin.

Detection:

Densitometric scanning was carried out using a CAMAG TLC Scanner-3 and LINOMAT-V at 254 nm and 366 nm in reflectance mode. The R_f values and peak areas of Quercetin in standard and sample tracks were recorded using WinCATS software, and quantification was carried out by comparing peak areas of the samples with those of the standard Quercetin

RESULTS AND DISCUSSION

Different solvent systems were evaluated to achieve optimal elution and resolution of phytoconstituents present in the herbal extracts [18,19]. The mobile phases tested included ethyl acetate: glacial acetic acid: formic acid: water (100:3:3:28), ethyl acetate: methanol: water: toluene (100:13:10:13), chloroform: ethyl acetate: methanol (6:4:0.3), toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4), and toluene: ethyl acetate (93:7). Among the five mobile phases examined, toluene: ethyl acetate: formic acid: methanol in the ratio of 3:6:1.6:0.4 provided superior resolution and better elution of marker compounds across all tested extracts. Therefore, this solvent system was selected as the optimized mobile phase for the detection and analysis of constituents in the herbal extracts. The chamber saturation time was optimized to 10 minutes at room temperature ($25 \pm 1^\circ\text{C}$). Densitometric scanning was carried out at 254 nm in reflectance mode. The R_f values of the marker compounds ranged from 0.08 to 0.91, indicating satisfactory separation. The detection and quantification of the marker compounds in the herbal raw material extracts are presented in Table 1. The Quercetin content was estimated as 0.1465% in *Curcuma domestica*, 0.3101% in *Alpinia galanga*,

0.0527% in *Elettaria cardamomum*, 0.1311% in *Gossypium arboreum*, and 0.1151% in *Diospyros ebenum*. Quercetin exerts diverse pharmacological effects through multiple molecular and cellular mechanisms. *Curcuma domestica* exhibits potent anti-inflammatory, antioxidant, antimicrobial, and anticancer activities, primarily mediated through modulation of the NF- κ B signaling pathway, inhibition of cyclooxygenase-2 (COX-2), suppression of pro-inflammatory cytokines, and activation of antioxidant defense mechanisms via the Nrf2 pathway [20,21]. *Alpinia galanga* possesses anti-inflammatory, antimicrobial, antioxidant, and gastroprotective activities, which are attributed to inhibition of nitric oxide production, downregulation of MAPK and NF- κ B signaling pathways, and free-radical scavenging effects of its flavonoids and phenolic constituents [22, 23]. *Elettaria cardamomum* demonstrates antioxidant, anti-inflammatory, antimicrobial, and cardioprotective activities, mainly through attenuation of oxidative stress, inhibition of lipid peroxidation, and modulation of inflammatory mediators such as TNF- α and interleukins via suppression of NF- κ B activation [24,25]. *Gossypium arboreum* exhibits anti-inflammatory, antimicrobial, wound-healing, and antioxidant activities, which are associated with inhibition of prostaglandin synthesis, scavenging of reactive oxygen species, and enhancement of cellular repair mechanisms through regulation of inflammatory and oxidative stress pathways [26,27]. *Diospyros ebenum* shows antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective activities, largely mediated by phenolic and flavonoid compounds that exert free-radical scavenging effects, inhibit lipid peroxidation, and modulate inflammatory signaling pathways such as NF- κ B and apoptotic cascades [28,29]. Table 1 shows the comparative HPTLC quantification of quercetin in selected medicinal plant extracts along with the standard. The R_f values of the marker in all extracts closely matched that of the quercetin standard (0.87–0.89), confirming the presence of quercetin. Among the samples, *Alpinia galanga* exhibited the highest quercetin content (0.310%), while *Elettaria cardamomum* showed the lowest (0.052%). The developed HPTLC method was found to be simple, rapid, cost-effective, and reproducible, enabling the simultaneous analysis of multiple samples with minimal solvent consumption. These features make



the method highly suitable for routine quality control and standardization of herbal raw materials as well as polyherbal formulations. The method generates characteristic chromatographic fingerprint profiles that can be effectively utilized for authentication and detection of adulteration. Overall, the results confirm

that quercetin can serve as a reliable phytochemical marker for the standardization of flavonoid-rich medicinal plants and establish HPTLC as an efficient analytical technique for Pharmacognostical evaluation and quality assurance of herbal medicines.

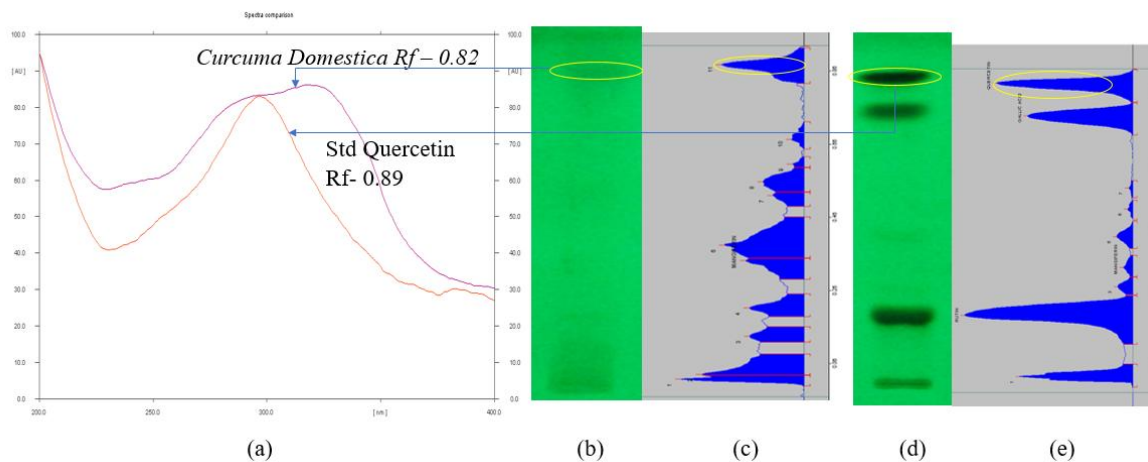


Figure 1: Chromatogram, Densitogram, and overlay profile of quercetin in Curcuma domestica

- a. Overlay profile of quercetin in Curcuma domestica
- b. Chromatogram of quercetin in Curcuma domestica
- c. Densitogram of quercetin in Curcuma domestica

- d. Chromatogram of quercetin standard
- e. Densitogram of quercetin standard

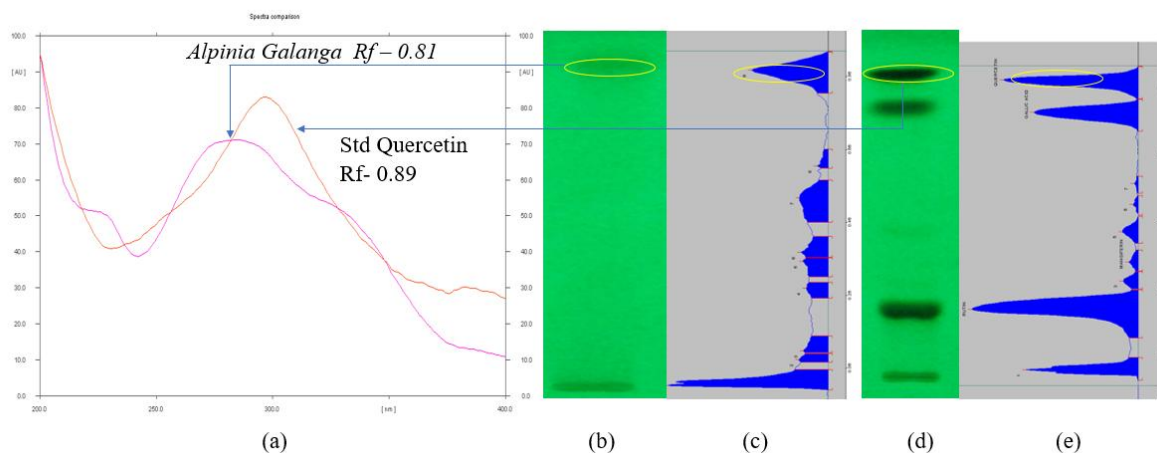


Figure 2: Chromatogram, Densitogram, and overlay profile of quercetin in Alpinia galanga

- a. Overlay profile of quercetin in Alpinia galanga
- b. Chromatogram of quercetin in Alpinia galanga
- c. Densitogram of quercetin in Alpinia galanga

- d. Chromatogram of quercetin standard
- e. Densitogram of quercetin standard

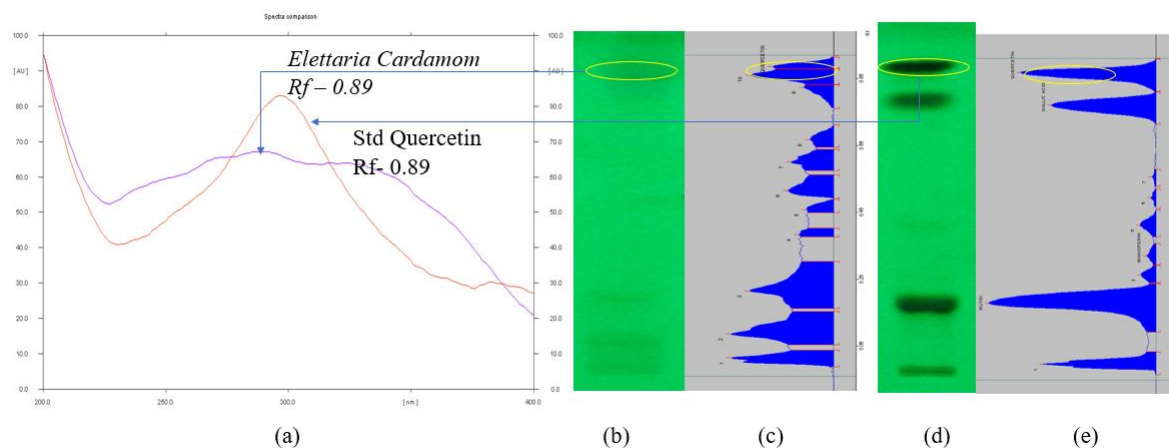


Figure 3: Chromatogram, Densitogram, and overlay profile of quercetin in Elettaria cardamomum

- a. Overlay profile of quercetin in Elettaria cardamomum
 b. Chromatogram of quercetin in Elettaria
 c. Densitogram of quercetin in Elettaria cardamomum
 d. Chromatogram of quercetin standard
 e. Densitogram of quercetin standard

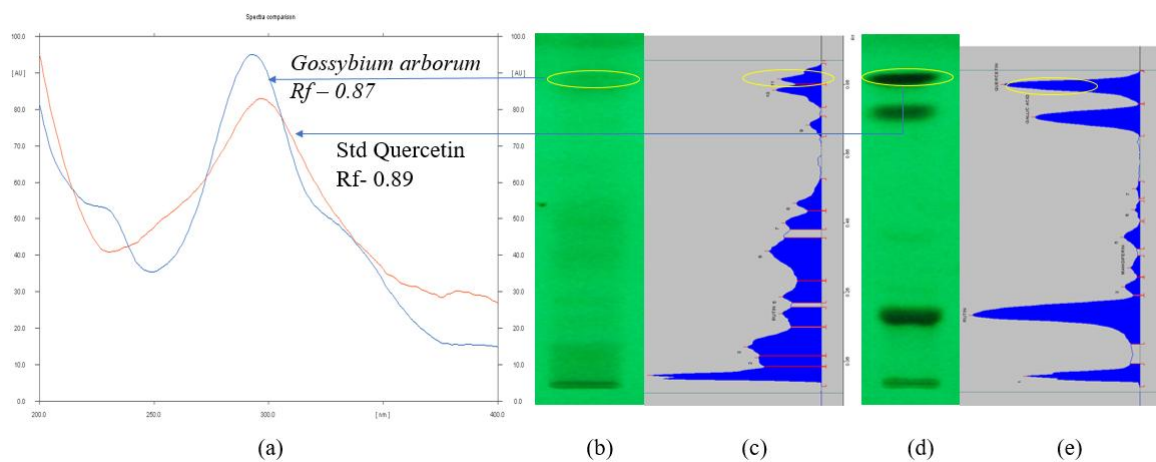


Figure 4: Chromatogram, Densitogram, and overlay profile of quercetin in Gossypium arboreum

- a. Overlay profile of quercetin in Gossypium arboreum
 b. Chromatogram of quercetin in Gossypium arboreum
 c. Densitogram of quercetin in Gossypium arboreum
 d. Chromatogram of quercetin standard
 e. Densitogram of quercetin standard

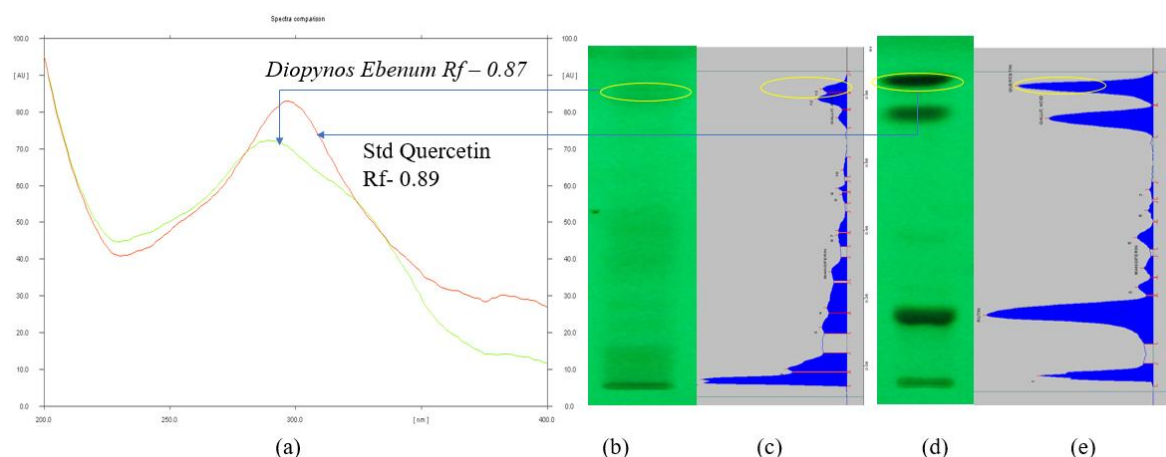


Figure 5: Chromatogram, Densitogram, and overlay profile of quercetin in Diospyros ebenum

- Overlay profile of quercetin in Diospyros ebenum
- Chromatogram of quercetin in Diospyros ebenum
- Densitogram of quercetin in Diospyros ebenum
- Chromatogram of quercetin standard
- Densitogram of quercetin standard

Sample No.	Plant Name	Rf Value of Marker in Extract	Rf Value of Quercetin Standard	Area of Standard Quercetin	Area of Marker in Extract	Amount of Marker ($\mu\text{g}/5 \mu\text{l}$ & $\mu\text{g}/8 \mu\text{l}$ of Extract / $5 \mu\text{l}$ of Standard)	% of Marker in Sample
1	Curcuma domestica	0.88	0.89	10817.7	2536.8	1.172	0.146%
2	Alpinia galanga	0.88	0.89	10817.7	5368.5	2.431	0.310%
3	Elettaria cardamomum	0.89	0.89	10817.7	913.4	0.422	0.052%
4	Gossypium arboreum	0.87	0.89	10817.7	2270.7	1.049	0.131%
5	Diospyros ebenum	0.88	0.89	10817.7	1993.2	0.9212	0.115%
6	Quercetin Standard	0.89	0.89	10817.7	10817.7	15	100%

Table 1. Quantitative estimation of quercetin in selected medicinal plant extracts

CONCLUSION

The present study successfully optimized an HPTLC method using toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4) as the mobile phase, which enabled efficient separation and reliable

quantification of quercetin in selected herbal raw materials. The developed method demonstrated good resolution, reproducible Rf values, and sensitive densitometric detection at 254 nm. Quantitative analysis revealed appreciable levels of quercetin in Curcuma domestica, Alpinia galanga, Elettaria



cardamomum, *Gossypium arboreum*, and *Diospyros ebenum*, supporting their reported antioxidant and anti-inflammatory potential. The well-documented pharmacological activities of quercetin, including antioxidant, anti-inflammatory, cardioprotective, neuroprotective, and antiviral effects, further emphasize the therapeutic relevance of these medicinal plants. Overall, the findings provide scientific validation for the traditional use of these botanicals in classical Siddha polyherbal formulations and establish HPTLC as a reliable tool for their quality control and standardization.

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REFERENCES

1. Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines – A review. *Int J Biodivers Conserv*. 2012;4(3):101–112.
2. World Health Organization. *Quality Control Methods for Herbal Materials*. Geneva: WHO; 2011.
3. Chaudhary SK, Singh A. Marker-based standardization of herbal drugs: An overview. *Pharmacogn Rev*. 2011;5(10):123–128.
4. Harborne JB, Williams CA. *Advances in flavonoid research since 1992*. *Phytochemistry*. 2000;55(6):481–504.
5. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. *Sci World J*. 2013; 162750.
6. Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells. *Pharmacol Rev*. 2000;52(4):673–751.
7. Boots AW, Haenen GR, Bast A. Health effects of quercetin: From antioxidant to nutraceutical. *Eur J Pharmacol*. 2008;585(2–3):325–337.
8. Russo M, Spagnuolo C, Tedesco I, Bilotto S, Russo GL. The flavonoid quercetin in disease prevention and therapy. *Biochem Pharmacol*. 2012;83(1):6–15.
9. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: Food sources and bioavailability. *Am J Clin Nutr*. 2004;79(5):727–747.
10. Reich E, Schibli A. *High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants*. Stuttgart: Thieme; 2007.
11. Sethi PD. *HPTLC: Quantitative Analysis of Pharmaceutical Formulations*. New Delhi: CBS Publishers; 2010.
12. Mukherjee PK. *Quality Control of Herbal Drugs*. 2nd ed. New Delhi: Business Horizons; 2007.
13. Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin. *Int J Biochem Cell Biol*. 2009;41(1):40–59.
14. Chudiwal AK, Jain DP, Somani RS. *Alpinia galanga*: An overview on phytopharmacological properties. *Indian J Nat Prod Resour*. 2010;1(2):143–149.
15. Bhandari P, Gupta AP. Pharmacognostic evaluation of *Elettaria cardamomum*. *J Pharmacogn Phytochem*. 2014;3(3):60–65.
16. Nadkarni KM. *Indian Materia Medica*. Vol. 1. Mumbai: Popular Prakashan; 2009.
17. Rauf A, Uddin G, Patel S, et al. *Diospyros species: Ethnopharmacology and phytochemistry*. *Biomed Pharmacother*. 2017; 93:714–730.
18. Rajasekaran A, Arivukkarasu R, Archana D. HPTLC method for estimation of gallic acid and quercetin in Haritaki – An Ayurvedic formulation. *Int J Pharm Tech Res*. 2011;3(2):986–999.
19. Arivukkarasu R, Bhuvanesh W, Dayanidhi C, Deepiga G, Dinesh D, Karunanithi M. Detection and estimation of quercetin and gallic acid in marketed green tea formulations by HPTLC technique. *World J Pharm Res*. 2023;12(3):690–697.
20. Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *Int J Biochem Cell Biol*. 2009;41(1):40–59.



21. Gupta SC, Patchva S, Aggarwal BB. Therapeutic roles of curcumin: lessons learned from clinical trials. *BioFactors*. 2013;39(1):1–13.
22. Matsuda H, Pongpiriyadacha Y, Morikawa T, Kishi A, Kataoka S, Yoshikawa M. Anti-inflammatory and antiallergic activities of galangin and related compounds from *Alpinia galanga*. *J Nat Med*. 2005;59(4):277–281.
23. Chudiwal AK, Jain DP, Somani RS. *Alpinia galanga* Willd. –an overview on phytopharmacological properties. *Pharmacogn Rev*. 2010;4(8):56–63.
24. Verma SK, Jain V, Katewa SS. Blood pressure lowering, fibrinolysis enhancing and antioxidant activities of cardamom (*Elettaria cardamomum*). *J Med Food*. 2009;12(4):732–739.
25. Al-Zuhair H, El-Sayeh B, Ameen HA, Al-Shoora H. Pharmacological studies of cardamom (*Elettaria cardamomum*). *J Ethnopharmacol*. 1996;52(3):165–171.
26. Sevukarajan M, Viswanathan S, Thirugnanasambantham P, Vijayasekaran V. Anti-inflammatory activity of *Gossypium arboreum* Linn. *Pharmacogn J*. 2010;2(11):428–430.
27. Kamboj VP. Herbal medicine. *Indian J Tradit Knowl*. 2012;11(3):333–344.
28. Mallavadhani UV, Panda AK, Rao YR. Pharmacology and chemotaxonomy of *Diospyros* species. *Phytochemistry*. 1998;49(4):901–951.
29. Lee JH, Lee KT, Yang JH, Baek NI. Antioxidant activity of phenolic compounds isolated from *Diospyros* species. *J Nat Prod*. 2001;64(2):203–206.

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