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COMPARATIVE HPTLC QUANTIFICATION OF RUTIN IN TRADITIONALLY USED MEDICINAL PLANT EXTRACTS

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ABSTRACT

Variability in phytochemical composition of herbal raw materials necessitates robust marker-based standardization to ensure quality, safety, and therapeutic consistency. Rutin, a bioactive flavonol glycoside, is widely used as a quality control marker in flavonoid-rich medicinal plants. The present study aimed to develop and apply a validated HPTLC method for the quantitative estimation of rutin in selected medicinal plant extracts commonly used in traditional systems of medicine. Methanolic extracts of *Azadirachta indica* (flowers and leaves), *Gossypium arboreum* (flowers), *Cassia auriculata* (bark), *Pachygone brevifolia* (leaves), *Cuminum cyminum* (fruits), and *Vitex trifolia* (leaves) were analyzed using HPTLC. Chromatographic separation was achieved on silica gel 60 F254 plates using toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4, v/v/v/v) as the mobile phase. Densitometric scanning was performed at 254 nm.**:** Rutin was well resolved with Rf values ranging from 0.16 to 0.20. Quantitative analysis revealed rutin content of 0.28% (*A. indica* flowers), 0.22% (*A. indica* leaves), 0.32% (*C. auriculata* bark), 0.01% (*G. arboreum* flowers), 0.33% (*P. brevifolia* leaves), and 0.13% (*V. trifolia* leaves). The HPTLC fingerprints demonstrated good resolution and reproducibility. The developed HPTLC method is simple, rapid, and reliable for the quantification of rutin in medicinal plant extracts. The findings support the use of rutin as a suitable phytochemical marker for quality control

and standardization of flavonoid-rich herbal raw materials and traditional polyherbal formulations.

KEYWORDS: *A. indica*, *G. arboreum*, *V. Trifolia*, Rutin and HPTLC.

INTRODUCTION

Herbal medicines continue to play a pivotal role in primary healthcare systems worldwide, particularly in developing countries, owing to their accessibility, cultural acceptance, and therapeutic efficacy. However, variability in phytochemical composition due to geographical origin, plant part used, harvesting conditions, and processing methods necessitates robust standardization strategies to ensure quality, safety, and consistency of herbal raw materials and finished formulations.[1,2] Marker-based standardization using chromatographic techniques has therefore become an essential component of Pharmacognostical evaluation. Rutin (quercetin-3-O-rutinoside) is a naturally occurring flavonol glycoside widely distributed in medicinal plants and dietary sources. It has gained significant attention as a phytochemical marker owing to its diverse pharmacological properties, including antioxidant, anti-inflammatory, Vaso protective, antidiabetic, cardioprotective, neuroprotective, and antiviral activities.[3–5] Owing to its stability and therapeutic relevance, rutin is frequently employed as a quality control marker for flavonoid-rich herbal drugs.[6] Plants such as *Azadirachta indica* (flowers and bark), *Gossypium arboreum* (leaves), *Cassia auriculata* (bark), *Pachygone brevifolia* (leaves), *Cuminum cyminum* (fruits), and *Vitex trifolia* (leaves) are well documented in traditional systems of medicine for their antioxidant, anti-infective, anti-inflammatory, antidiabetic, and hepatoprotective properties.[7–12] The therapeutic efficacy of these plants has been largely attributed to their polyphenolic and flavonoid constituents, among which rutin is a major bioactive component. High-Performance Thin-Layer Chromatography (HPTLC) is a validated, cost-effective, rapid, and reproducible analytical technique recommended by pharmacopoeias and regulatory authorities for the qualitative and quantitative evaluation of herbal drugs. It allows simultaneous analysis of multiple samples with minimal solvent consumption and provides characteristic chromatographic fingerprints essential for quality control.[13,14] In the present study, HPTLC was employed for the quantification of rutin in selected medicinal plant extracts to establish marker-based standardization and support their Pharmacognostical authentication.

MATERIALS AND METHODS

Collection of raw materials for HPTLC screening

The raw materials were received as gift sample from Traditional siddha medical practitioner Mr. Cinnathambi, Boomidi, Dharmapuri district and confirmed the raw materials with 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—*Azadirachta indica* (flowers and bark), *Gossypium arboreum* (leaves), *Cassia auriculata* (bark), *Pachygone brevifolia* (leaves), *Cuminum cyminum* (fruits), and *Vitex trifolia* (leaves)—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 100µl syringe and a Camag Linomat V applicator, the herbal formulation solutions were spotted as 4 mm wide bands on recoated plate 60 F254 (10 cm × 10 cm with 0.2 mm m thickness, E. Merck). The dimensions of the slit were maintained at 6 x 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 µL (or 5 µL where specified) of sample extracts were applied. The migration distance was maintained at 80 mm. Plates were

air-dried prior to development. Chromatographic development was carried out in a CAMAG twin-trough glass chamber (10 × 10 cm) pre-saturated for 10 min at 25 ± 1°C and 40% relative humidity with the mobile phase consisting of toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4, v/v/v/v). Plate development was performed up to a distance of 8 cm from the bottom.

Detection

Densitometric scanning was performed using a CAMAG TLC Scanner-3 and LINOMAT-V at 254 nm and 366 nm in reflectance mode. The Rf values and peak areas of rutin 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 rutin.

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 ± 1 °C). Densitometric scanning was carried out at 254 nm in reflectance mode. The Rf 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 rutin content was estimated as 0.28% in *Azadirachta indica* flower extract, 0.22% in *Azadirachta indica* leaf extract, 0.32% in *Cassia auriculata* bark extract, 0.01% in *Gossypium arboreum* flower extract, 0.33% in *Pachygone brevifolia* leaf extract, and 0.13% in *Vitex trifolia* leaf extract.Rutin exerts diverse pharmacological effects through multiple molecular and cellular mechanisms. Its antioxidant activity is primarily attributed to direct scavenging of reactive oxygen and nitrogen species, chelation of transition metal ions, and up-regulation of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase via activation of the Nrf2 signaling pathway.[3,15] The anti-inflammatory activity

of rutin is mediated through inhibition of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukins (IL-1β and IL-6), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS), largely through suppression of NF-κB and MAPK signaling pathways.[4,16] These mechanisms account for its therapeutic relevance in inflammatory disorders, arthritis, and chronic inflammatory diseases. In cardiovascular and metabolic disorders, rutin improves endothelial function, reduces capillary fragility, inhibits low-density lipoprotein oxidation, and modulates lipid metabolism. Additionally, rutin exhibits antidiabetic activity by enhancing insulin sensitivity, inhibiting α-glucosidase activity, and reducing oxidative stress-induced pancreatic β-cell damage.[5,17] Neuroprotective effects of rutin have also been reported, including attenuation of neuroinflammation, inhibition of acetylcholinesterase activity, and prevention of neuronal apoptosis through modulation of mitochondrial pathways, highlighting its potential in neurodegenerative disorders such as Alzheimer's and Parkinson's diseases.[20] Furthermore, rutin demonstrates antiviral and immunomodulatory properties by interfering with viral replication, protease activity, and host cell entry mechanisms, along with regulation of cytokine responses, making it relevant in respiratory viral infections and emerging viral diseases.[21,22] The HPTLC profiles and quantified antioxidant markers obtained from *Azadirachta indica* (flowers and bark), *Gossypium arboreum* (leaves), *Cassia auriculata* (bark), *Pachygone brevifolia* (leaves), *Cuminum cyminum* (fruits), and *Vitex trifolia* (leaves) correlate well with their reported pharmacological activities. These findings support their therapeutic relevance in classical Siddha polyherbal formulations. *Azadirachta indica* (Neem) flowers and leaves are traditionally valued for their medicinal properties and are incorporated as a key ingredient (10%) in Pancha Karpa Kuliyal Chooranam, along with Seeraga Chooranam (q.s.), Madhumega Kudineer Chooranam (16.66%), and Sarva Sura Kudineer Chooranam (70%).Similarly, *Gossypium arboreum* flowers and leaves are traditionally employed in Siddha medicine and are included as constituent ingredients in formulations such as Poorana Chandirodhaya Chendooram (q.s.) and Dhathu Kalpa Legiyum (q.s.), contributing to their therapeutic efficacy. *Cassia auriculata* flowers and leaves are incorporated into polyherbal preparations containing Pancha Kuliyal Chooranam (6.25%), Multanimatti Chooranam (25%), Naththai Choori Chooranam (25%), and Avarai Kudineer Chooranam (16.66%), collectively enhancing the therapeutic potential of these formulations.Overall, the quantified levels of rutin in *Azadirachta indica*, *Cassia auriculata*, *Pachygone brevifolia*, *Vitex trifolia*, and *Gossypium arboreum* extracts underscore their contribution to antioxidant and anti-

inflammatory activities and provide scientific validation for their inclusion in traditional Siddha polyherbal formulations.

Sample number	Name/Amount of sample in µl	Rf values of marker in extracts	Rf value of Rutin	Area of standard Rutin marker	Area of marker in extracts	Amount of marker present in µg/ 5 µl & µg/ 8 µl of extracts / 5 µl of standards	Percentage of marker present in sample
1	*Azadirata indica* flower extract (8µl)	0.17	0.16	22061.8	10016.2	2.27 µg	0.28%
2	*Azadirata indica* leaf extract (8µl)	0.18	0.16	22061.8	8116.6	1.83µg	0.22%
3	*Cassia auriculata* bark extract(8µl)	0.19	0.20	26135.1	13504.9	2.58µg	0.32%
4	*Gossypium arborum flower extract* (8µl)	0.20	0.20	21777.4	654.7	0.15µg	0.01%
5	*Pachygone brevifolia leaf extract* (8µl)	0.20	0.20	26135.1	13848.8	2.64µg	0.33%
6	*Vitex trifolia leaf extract* (5µl)	0.19	0.17	20020.8	2688.5	0.67µg	0.13%
7	*Standard Marker Rutin* (5µl)	0.17	-----	22061.8	-----	5µg	100%
		0.19	-----	26135.1	-----	5µg	100%
		0.20	-----	21777.4	-----	5µg	100%
		0.19	-----	20020.8	-----	5µg	100%

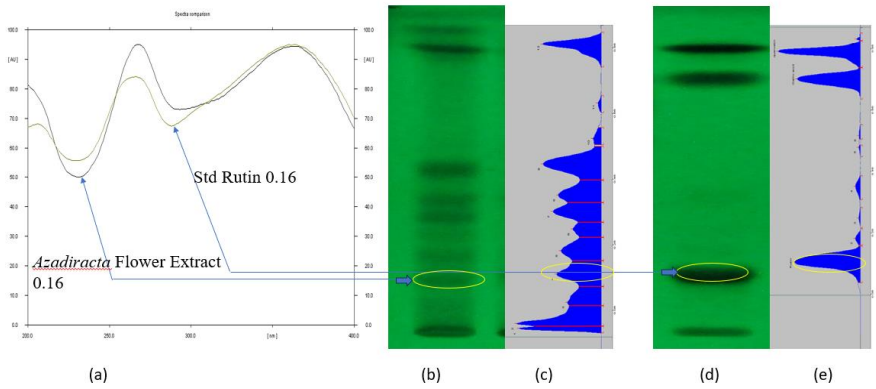

Figure: 1 Chromatogram, Densitogram and Overlay of rutin in *Azadiracta* flower extract.

a) Overlay of rutin in *Azadiracta* flower extract
b) Chromatogram of rutin in *Azadiracta* flower extract
c) Densitogram of rutin in *Azadiracta* flower extract
d) Chromatogram of rutin in *Azadiracta* flower standard
e) Densitogram of rutin in *Azadiracta* flower standard.

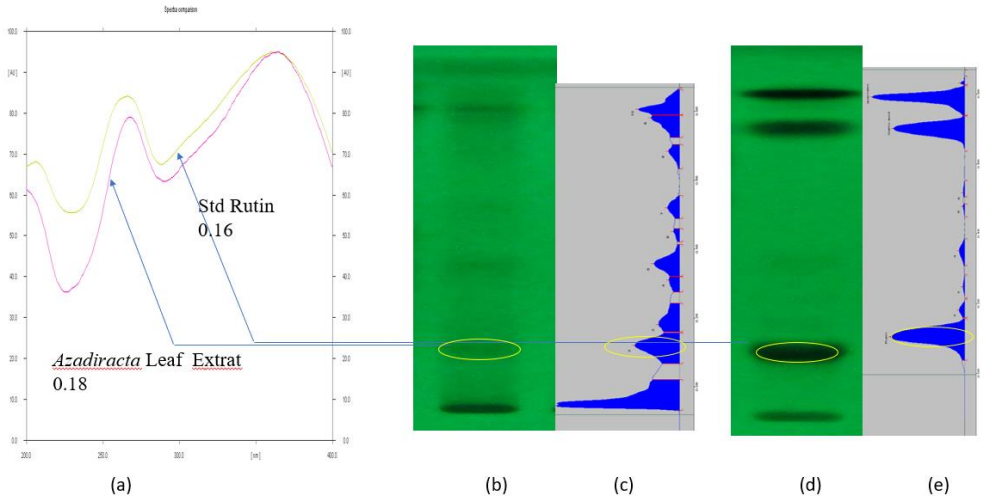

Figure: 2 Chromatogram, Densitogram and Overlay of rutin in *Azadiracta* Leaf extrat.

a) Overlay of rutin in *Azadiracta* Leaf extrat
b) Chromatogram of rutin in *Azadiracta* Leaf extrat
c) Densitogram of rutin in *Azadiracta* Leaf extrat
d) Chromatogram of rutin in *Azadiracta* Leaf standard
e) Densitogram of rutin in *Azadiracta* Leaf standard

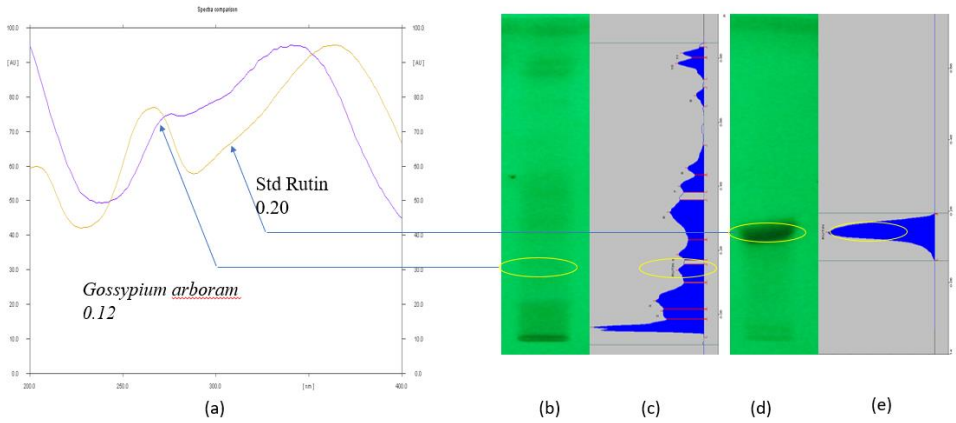

Figure: 3 Chromatogram, Densitogram and Overlay of rutin in *Gossypium arboram*.

a) Overlay of rutin Gossypium *arboram* extrat
b) Chromatogram of rutin in Gossypium *arboram* extrat
c) Densitogram of rutin in Gossypium *arboram* extrat
d) Chromatogram of rutin in Gossypium *arboram* standard
e) Densitogram of rutin in Gossypium *arboram* standard.

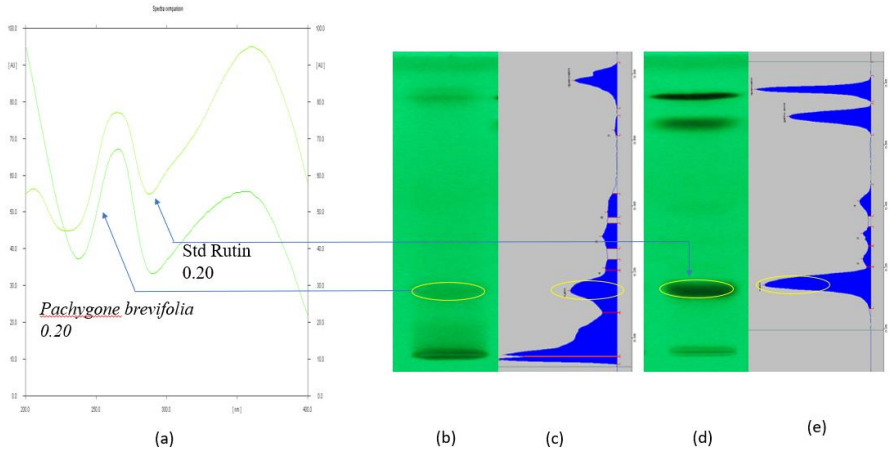

Figure: 4 Chromatogram, Densitogram and Overlay of rutin in *Pachygone brevifolia.*

a) Overlay of rutin Gossypium *arboram* extrat
b) Chromatogram of rutin in Gossypium *arboram* extrat
c) Densitogram of rutin in Gossypium *arboram* extrat
d) Chromatogram of rutin in Gossypium *arboram* standard
e) Densitogram of rutin in Gossypium *arboram* standard

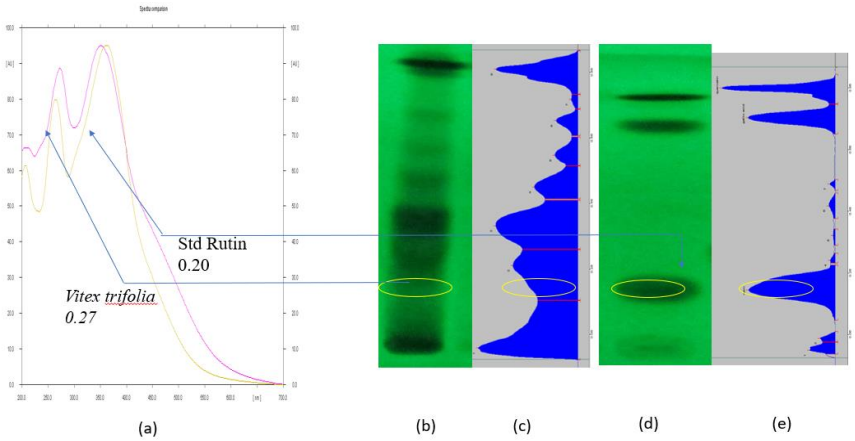

Figure: 5 Chromatogram, Densitogram and Overlay of rutin in *Cassia auriculata* bark

a) Overlay of rutin *Cassia auriculata* bark extract
b) Chromatogram of rutin in *Cassia auriculata* bark extract
c) Densitogram of rutin in *Cassia auriculata* bark extract
d) Chromatogram of rutin in *Cassia auriculata* bark standard
e) Densitogram of rutin in *Cassia auriculata* bark standard.

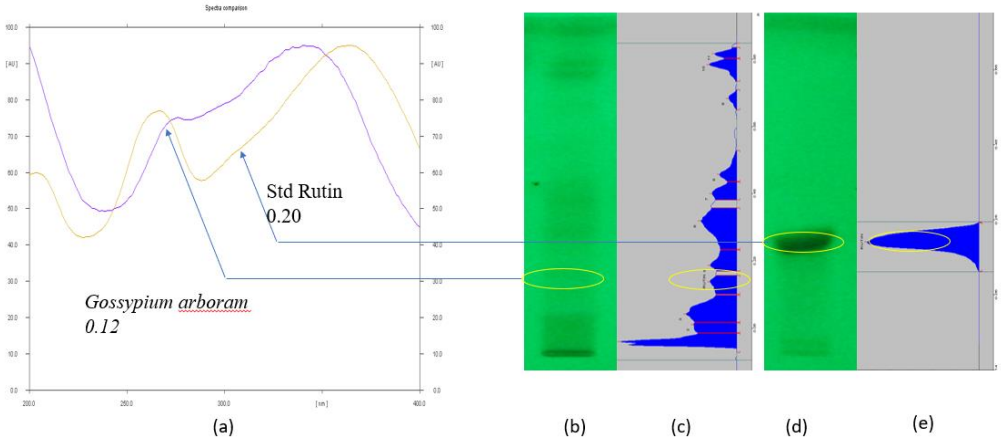

Figure: 6 Chromatogram, Densitogram and Overlay of rutin in *Cuminum cyminum*.

a) Overlay of rutin *Cuminum cyminum* extrat
b) Chromatogram of rutin in *Cuminum cyminum* extrat
c) Densitogram of rutin in *Cuminum cyminum* extrat
d) Chromatogram of rutin in *Cuminum cyminum* standard
e) Densitogram of rutin in *Cuminum cyminum* standard

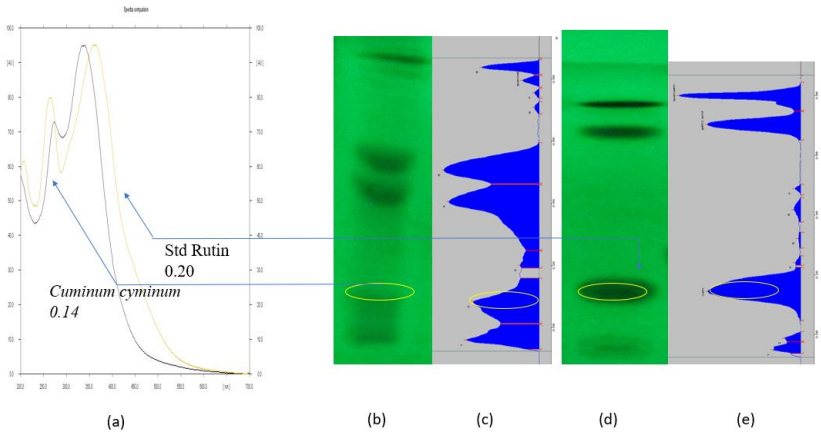

Figure: 7 Chromatogram, Densitogram and Overlay of rutin in *Vitex trifolia.*

a) Overlay of rutin *Vitex trifolia* extrat
b) Chromatogram of rutin in *Vitex trifolia* extrat
c) Densitogram of rutin in *Vitex trifolia* extrat
d) Chromatogram of rutin in *Vitex trifolia* standard
e) Densitogram of rutin in *Vitex trifolia* standard.

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, providing efficient separation and reliable quantification of rutin in selected herbal raw materials. The validated method demonstrated satisfactory resolution, reproducible Rf values, and effective densitometric detection at 254 nm. Quantitative analysis revealed appreciable levels of rutin in *Azadirachta indica*, *Cassia auriculata*, *Pachygone brevifolia*, and *Vitex trifolia*, supporting their antioxidant and anti-inflammatory potential. The pharmacological relevance of rutin, including antioxidant, anti-inflammatory, cardioprotective, neuroprotective, and antiviral activities, substantiates the therapeutic significance of these medicinal plants. Overall, the findings provide scientific validation for the traditional use of these botanicals in classical Siddha polyherbal formulations and support their quality control and standardization using HPTLC techniques.

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