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RESEARCH ARTICLE

ECO-FRIENDLY HPTLC DEVELOPMENT AND DETERMINATION OF PHYTOCHEMICALS LIKE BAICALEIN, BETASITOSTEROL, APIGENIN, ALLOIN, PROANTHOCYANIDIN, GALLIC ACID, QUERCETIN, LUPIOL AND VALIDATION OF BAICALEIN IN OROXYLUM INDICUM ROOT

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Abstract: Oroxylum Indicum kurz root, a member of the Bignoniaceae family, is a substantial source of phytosterol chemicals, including Baicalein. The plant traditionally used in Maharashtra, India, for as an astringent, an anti-inflammatory agent, an antioxidant agent, an anti-bronchitic agent, an anti-helminthic agent and an anti-microbial agent. The absence of standardization for Oroxylum Indicum (L.) kurz root and extracts determination of Phytochemicals like Betasitosterol, Apigenin, Alloin, Proanthocyanidin and Validation of Baicalein in Oroxylum Indicum Root. This study aims to develop a straight forward, novel, dependable, and precise HPTLC technique for quantifying Baicalein in Oroxylum Indicum root vent extract. The isolation and measurement of Baicalein were performed using HPTLC plates covered with Silica gel 60 F254 as the stationary phase. An HPTLC technique was established using Toluene: ethyl acetate: acetic acid in a ratio of 6:3:2 (v/v/v) as the optimal mobile phase. TLC plates at 335nm revealed a singular phytochemical with an Rf value of 0.54±2.21. The approach demonstrated selectivity for Baicalein in the extract of Oroxylum Indicum root, as shown by the overlay of UV spectra. The technique was verified for linearity, precision, accuracy, Limit of Detection (LOD), and Limit of Quantification (LOQ). It exhibited linear calibration curves (100-500 ng/band, r^2>0.995), % RSD < 2%, LOD and LOQ of 0.908107127 and 2.751839802, respectively, and a recovery rate of 78.54667 %. The methodology demonstrated robustness, with a relative standard deviation percentage of under 5%. A straightforward and sensitive HPTLC approach was validated for the detection of Baicalein in the extract of Oroxylum Indicum root, providing advantages in terms of time and cost-effectiveness.

Keywords: Baicalein, Betasitosterol, Apigenin, Alloin, Proanthocyanidin, Gallic Acid, Quercetin, Lupiol, Oroxylum Indicum root, High-performance thin-layer chromatography, validation.

INTRODUCTION

A simple, reliable, accurate, precise, and specific high-performance thin-layer chromatography (HPTLC) –densitometry method was developed and validated for the quantification of baicalin, a significant bioactive phytosterol, in *Oroxylum Indicum* root. *Oroxylum indicum*, which is commonly called Indian trumpet tree, *oroxylum*, Indian trumpet flower, broken bones, scythe tree, tree of Damocles, or midnight horror. It can reach a height of 18 metres (59 ft). Various segments of the tree are used in traditional plant in the *Bignoniaceae* family with various therapeutic potential such as as an astringent, an anti-inflammatory agent, an antioxidant agent, an anti-bronchitic agent, an anti-helminthic agent and an anti-microbial agent. The ethanolic *Oroxylum Indicum* root extract was prepared using soxhlet extraction method. This extract was screened for preliminary qualitative tests, and based on the screening results, secondary metabolites such as flavonoids, phenols, steroids, terpenoids, and tannins were further quantified. Oroxylum indicum contains a broad variety of phytochemicals such as tannins, alkaloids, saponins, sterols, flavonoids, lignins, glycosides, phenols, fats and oils. The active constituent of the O. indicum root is baicalin. Baicalin is also the main ingredient of flavonoid, which is an approved medical food and classified under Generally Recognized as Safe (GRAS) category by the United States Food and Drug Administration (USFDA). Antioxidant potential was assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH), Anti-inflammatory activity was evaluated through protein denaturation Assay, Additionally, an HPTLC method was developed and validated for baicalin quantification. The mobile phase of Toluene: ethyl acetate: acetic acid in a ratio of 6:3:2 (v/v/v) was used for achieving good separation. Densitometric determination was carried out at 335 nm. The calibration curves were found to be linear in the range between 200 and 1000 ng per spot. The developed method of HPTLC was validated for specificity, accuracy, precision and linearity. The ethanolic extract has unveiled significant antioxidant activity with a percentage inhibition of 67.34%.

Fig. 1 : Baicalein 3D structure

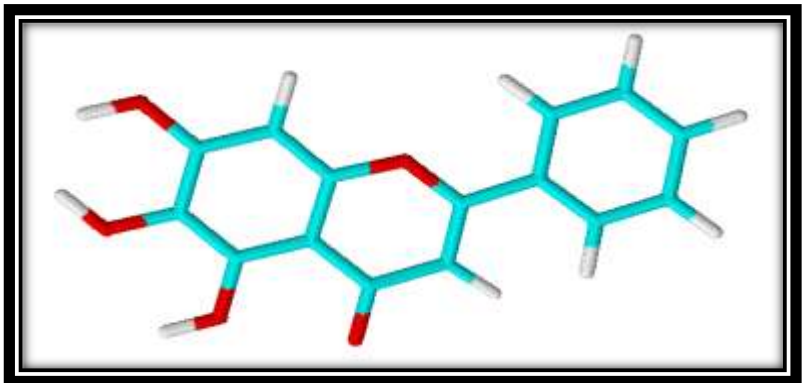


Fig. 2 : Baicalein 2D structure

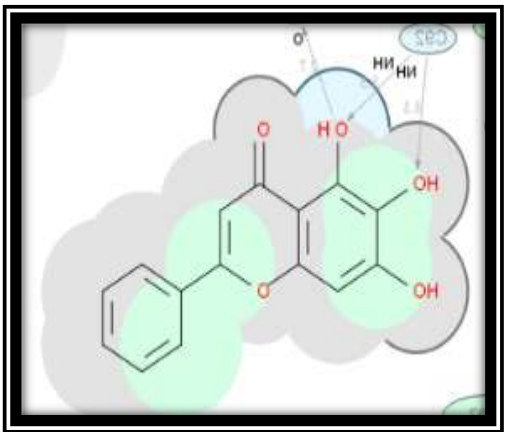
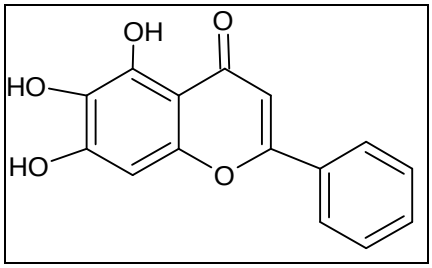


Fig. 3: The chemical structure of baicalein



METHODOLOGY & MATERIALS

Plant material

21st January 2023, the fresh foliage of the *Oroxylum indicum*(L.) kurz plant were collected from the Saundal village (16.70, 73.62) is situated in Rajapur Tehsil, within the Ratnagiri district of Maharashtra, India. The specimen, with the certification account number MMM001, was obtained from the Abasaheb Marathe Arts & New Commerce, Science College, Rajapur, District. Ratnagiri. Then plant Roots were first cut into small, uniform pieces to ensure even drying. These pieces were then spread out under shade, away from direct sunlight. Shade drying helped preserve the natural color, odour, and medicinal properties of the roots by preventing heat damage and loss of volatile compounds. Once fully dried root bark of *Oroxylum indicum* was air-dried and powdered pass-through sieve no. 40.

Preparation of extract

In a Soxhlet apparatus, 100 g of dried root powder was extensively defatted with ethanol for 24 hr at 40°C. The Soxhlet extraction process of *Oroxylum indicum* involved using its dried and powdered root bark as the starting material. A measured quantity 100 g of the dried root powder was placed in a thimble and loaded into the Soxhlet apparatus. Ethanol, commonly used as the solvent due to its efficiency in extracting bioactive compounds, was heated to produce vapors that condensed and repeatedly washed over the plant material. This continuous cycle for 24 hr. allowed for thorough extraction of phytochemicals such as flavonoids, phenolic, and other medicinal constituents. After several hours of extraction, the solvent containing a dark brownish material dissolved compounds was collected and evaporated to obtain a concentrated crude ethanolic extract. This extract was then stored for further HPTLC analysis or use in Antioxidant Potential studies.

EXPERIMENTAL WORK

HPTLC ANALYSIS & METHOD DEVELOPMENT

Selection of HPTLC Plate

The study employed pre-coated silica gel G 60 F254 HPTLC plates for the chromatographic separation with dimensions of 20 × 10 cm, 10 × 10 cm and 5 × 10 cm.

Activation of Pre-Coated Plate

Since the silica gel G 60 F254 plates are pre-coated, activation was required prior to sample application. Plates were activated at 110°C in a dry heat oven for 10 min prior to utilization.

Preparation of standard solution Baicalein

The stock solutions of standard solution of baicalein (1 mg/mL) were meticulously prepared utilizing Analytical Reagent (AR) grade ethanol.

Preparation of standard solution Apigenin

The stock solutions of standard solution of Apigenin (1 mg/mL) were meticulously prepared utilizing Analytical Reagent (AR) grade ethanol.

Preparation of standard solution Aloin

The stock solutions of standard solution of aloin (1 mg/mL) were meticulously prepared utilizing Analytical Reagent (AR) grade ethanol.

Preparation of standard solution Beta sitosterol

The stock solutions of standard solution of Beta sitosterol (1 mg/mL) were meticulously prepared utilizing Analytical Reagent (AR) grade ethanol.

Preparation of standard solution proanthocyanidin

The stock solutions of standard solution of proanthocyanidin (1 mg/mL) were meticulously prepared utilizing Analytical Reagent (AR) grade ethanol.

Preparation of test/sample solution

The stock solutions of test/sample solution *Oroxylum indicum(L.) kurz* root bark extract (10 mg/ml) were meticulously prepared utilizing Analytical Reagent (AR) grade ethanol.

Application of Sample

Using the CAMAG LINOMAT 5 applicator, the standard solution and sample solutions were loaded sample bands applied on the pre- coated plate along designated tracks. Bands were spotted 8 mm from the bottom and measured 8 mm in length on a plate of dimensions 20 × 10 cm, 10 × 10 cm and 5 × 10 cm. The sample volume was adjusted based on the volatility of the solvent, and a sharp band was obtained with 2 µl, 4 µl of sample application bands by using a 100 µl syringe with the assistance of a CAMAG ATS 4 (Muttenz, Switzer land) automatic applicator with an application Speed of 150 nl/s in the presence of nitrogen gas. The narrow interspaces between the bands were 10 mm. The densitometric study was carried out using CAMAG TLC Scanner 4 with vision CATS software (version 4.0.24032.1) at a wavelength of 335 nm in reflectance mode by a Deuterium lamp. The slit dimension of 5 x 0.2 mm micro was used for scanning with a scanning rate of 20 ms/S.

Mobile Phase Preparation and Chamber Saturation

The HPTLC plates were developed utilizing a mobile phase composed of Toluene: ethyl acetate: acetic acid in a ratio in a volumetric ratio of 6:3:2 (V/V/V)

Development of Spots

A glass CAMAG® Twin Trough Chamber (TTC) 10 × 10 cm was utilized to saturate the mobile phase for a duration of 20 min. Samples were deposited onto HPTLC plates utilizing a sample applicator equipped with a 100 µL microliter Syringe (ILS) and controlled nitrogen flow. The mobile phase, comprising Toluene: ethyl acetate: acetic acid in a ratio in a volumetric ratio of 6:3:2 (V/V/V), was freshly prepared and poured into a clean, dry twin trough chamber. The chamber was then allowed to saturate for 20 minutes before use. The plate was placed in a twin-trough critical glass chamber (CAMAG) pre-saturated with the mobile phase consisting of Toluene: ethyl acetate: acetic acid in a ratio in a volumetric ratio of 6:3:2 (V/V/V) and removed when the solvent reached up to 70 mm. After the development process, To recognize the bands, the plate was further dried by using hair dryer for 5 min and examined under UV light. The observed spots were sharp with no tailing, indicating a good separation and the R_f values for each band were recorded

Rf Value Calculation

The Rf value was calculated using the formula:
Rf = Distance travelled by the compound ÷ Distance travelled by the solvent front, with both measurements taken from the point of origin.

Development of a solvent system

Fig. 4 : Twin Trough Chamber for development of spot

Track Assignment and Mobile Phase

for Baicalein

Table 1: Track Assignment for **Baicalein**

Track Assignment

Track	Vial ID	Description	Volume	Type
1	RCP_ oroxylum I ndicum Extract	RCP_ oroxylum Indicum root Extract	2.0 µL	Sample
2	RCP_ Baicalein	RCP_ Baicalein	4.0 µL	Reference
3	RCP_ oroxylum I ndicum Extract	RCP_ oroxylum Indicum root Extract	4.0 µL	Sample

For the elution, diverse solvent systems were utilized- Water : Formic Acid : Acetone : Ethyl Acetate (0.5:1:2:10, V/V/V/V), Water : Formic Acid : Acetone : Ethyl Acetate (2:1:2:10, V/V/V/V), Toluene : Ethyl Acetate : Formic Acid (7:2:1, V/V/V), Toluene : Ethyl Acetate : Acetic Acid (7.25:3.75:0.5, V/V/V), N-hexane: Ethyl Acetate : Acetic Acid (6.5:3.5:0.2, V/V/V), Toluene : Ethyl Acetate : Formic Acid : Acetone (6.5:2:1:0.5, V/V/V/V), Toluene : Ethyl Acetate : Formic Acid : Acetone (6.5:2:1:1.5, V/V/V/V), Toluene : Ethyl Acetate : Formic Acid (6:2:1, V/V/V), Toluene : Ethyl Acetate : Formic Acid (6:3:1, V/V/V), Toluene : Ethyl Acetate : Formic Acid (6:3:2, V/V/V). The solvent system consisting of Toluene: Ethyl Acetate: Formic Acid (6:3:2, V/V/V) was produced good results and this mobile phase selected for HPTLC analysis.

Track Assignment and Mobile Phase for Alloin

The solvent system consisting of Chloroform: Ethyl acetate (7.5:2.5 ml) (V/V) was produced good results and this mobile phase selected for HPTLC analysis.

Table 2: Track Assignment for **Alloin**

Track Assignment

Track	Vial ID	Description	Volume	Type
1	RCP_Oroxylum In dicum plant extra ct	RCP_ oroxylum Indicum root Extract	2.0 µL	Sample
2	RCP_alloin	RCP_ Alloin	4.0 µL	Reference
3	RCP_Oroxylum In dicum plant extra ct	RCP_ oroxylum Indicum root Extract	4.0 µL	Sample

Track Assignment and Mobile Phase for Apigenin

The solvent system consisting of Toluene: Ethyl acetate: Formic acid (6:4:0.2 ml) (V/V/V) was produced good results and this mobile phase selected for HPTLC analysis.

Table 3: Track Assignment for **Apigenin**

Track Assignment

Track	Vial ID	Description	Volume	Type
1	RCP_Oroxylum In dicum plant extra ct	RCP_Oroxylum Indicum root extract	2.0 µL	Sample
2	RCP_Marker apig enin	RCP_Apigenin	2.0 µL	Reference
3	RCP_Oroxylum In dicum plant extra ct	RCP_Oroxylum Indicum root extract	4.0 µL	Sample

Track Assignment and Mobile Phase for Proanthocyanidine

The solvent system consisting of Toluene: Ethyl acetate: Formic acid (7:2:1 ml) (V/V/V) was produced good results and this mobile phase selected for HPTLC analysis.

Table 4: Track Assignment for **Proanthocyanidine**

Track Assignment

Track	Vial ID	Description	Volume	Type
1	RCP_Oroxylum Indicum plant extract	RCP_Oroxylum Indicum root extract	2.0 µL	Sample
2	RCP_1Proanthocynidine	RCP_1Proanthocynidine	4.0 µL	Reference
3	RCP_Oroxylum Indicum plant extract	RCP_Oroxylum Indicum root extract	4.0 µL	Sample

Track Assignment and Mobile Phase for Betasitosterol

The solvent system consisting of Toluene: Ethyl acetate : Formic acid (7:3:1 ml) (V/V/V) was produced good results and this mobile phase selected for HPTLC analysis.

Table 5: Track Assignment for **Betasitosterol**

Track Assignment

Track	Vial ID	Description	Volume	Type
1	RCP_ oroxylum Indicum root Extract	RCP_ oroxylum Indicum root Extract	2.0 µL	Sample
2	RCP_Betasitosterol	RCP_Betasitosterol	4.0 µL	Reference
3	RCP_ oroxylum Indicum root Extract	RCP_ oroxylum Indicum root Extract	4.0 µL	Sample

Track Assignment and Mobile Phase for Gallic Acid

The solvent system consisting of Toluene: Ethyl acetate : Formic acid: Methanol (4:4:2:2 ml) (V/V/V) was produced good results and this mobile phase selected for HPTLC analysis.

Table 6: Track Assignment for **Gallic Acid**

Track Assignment

Track	Vial ID	Description	Volume	Type
1	extract	RCP_Oroxylum Indicum root extract	2.0 µL	Reference
2	Standard Gallic acid	RCP_standard Gallic acid	4.0 µL	Reference
3	extract f	RCP_Oroxylum Indicum root extract	2.0 µL	Reference

Track Assignment and Mobile Phase for Quercetin

The solvent system consisting of Toluene: Ethyl acetate: Formic acid (5:4:1 ml) (V/V/V) was produced good results and this mobile phase selected for HPTLC analysis.

Table 7: Track Assignment for **Quercetin**

Track Assignment

Track	Vial ID	Description	Volume	Type
1	Extract	RCP_Oroxylum Indicum root extract	4.0 µL	Sample
2	Quercetin	Quercetin	3.0 µL	Reference
3	Extract	RCP_Oroxylum Indicum root extract	3.0 µL	Sample

Track Assignment and Mobile Phase for Lupiol

The solvent system consisting of Glacial Acetic Acid : Methanol : Toluene (0.1:1:9 ml) (V/V/V) was produced good results and this mobile phase selected for HPTLC analysis.

Table 8: Track Assignment for **Lupiol**

Track Assignment

Track	Vial ID	Description	Volume	Type
1	RCP_ Extract	RCP_Oroxylum Indicum root extract	2.0 µL	Sample
2	Marker lupiol	RCP_lupiol	4.0 µL	Reference
3	RCP_ Extract	RCP_Oroxylum Indicum root extract	4.0 µL	Sample

Baicalein Method validation

Linearity

The Baicalein and *Oroxylum indicum* (L.) kurz root extract sample was applied (1-5 µl/band) to a HPTLC plate in distinct working solutions, with each band ranging from 100–500 ng/spot. After development, area was recorded and correlation coefficient, slope and intercept were calculated from the linearity graph.

Table 9: Track Assignment for **Linearity**

Track Assignment

Track	Vial ID	Description	Volume	Type
1	Baicalein marker	Baicalein marker 1mg/ml	1.0 µL	Reference
2	Baicalein marker	Baicalein marker 1mg/ml	2.0 µL	Reference
3	Baicalein marker	Baicalein marker 1mg/ml	3.0 µL	Reference
4	Baicalein marker	Baicalein marker 1mg/ml	4.0 µL	Reference
5	Baicalein marker	Baicalein marker 1mg/ml	5.0 µL	Reference
6	Oroxylum indicu m extract	Oroxylum indicum extract	2.0 µL	Sample
7	Oroxylum indicu m extract	Oroxylum indicum extract	2.0 µL	Sample
8	Oroxylum indicu m extract	Oroxylum indicum extract	2.0 µL	Sample

Track Assignment notes

Accuracy

Accuracy was assessed by spiking method was adding a known amount of a standard substance Baicalein (2.0 µl of 1 mg/ml) to a sample *Oroxylum indicum*(L.) kurz root extract

Table 10: Track Assignment for **Accuracy**

Track Assignment

Track	Vial ID	Description	Volume	Type
1	Baicalein marker	Baicalein marker	2.0 µL	Reference
2	Baicalein marker	Baicalein marker	2.0 µL	Reference
3	Baicalein marker	Baicalein marker	2.0 µL	Reference
+	Oroxylum indicu m extract	Oroxylum indicum extract	2.0 µL	Sample
4	Baicalein marker	Baicalein marker	2.0 µL	Reference
+	Oroxylum indicu m extract	Oroxylum indicum extract	2.0 µL	Sample
5	Baicalein marker	Baicalein marker	2.0 µL	Reference
+	Oroxylum indicu m extract	Oroxylum indicum extract	2.0 µL	Sample
6	Baicalein marker	Baicalein marker	2.0 µL	Reference
+	Oroxylum indicu m extract	Oroxylum indicum extract	2.0 µL	Sample
7	Oroxylum indicu m extract	Oroxylum indicum extract	2.0 µL	Sample
8	Oroxylum indicu m extract	Oroxylum indicum extract	2.0 µL	Sample

Track Assignment notes

Precision

Intraday precision

Intraday precision was assessed by applying six replicates of each marker at three distinct concentrations on an HPTLC plate. This study of intraday precision involves testing each concentration six times across various days.

Repeatability

Repeatability was determined using 2 µL of 100 µg/ml Baicalein solutions, applied six times using a CAMAG LINOMAT 5 applicator.

Intermediate precision (Inter-Day)

Inter-day precision was evaluated by applying 100 ng, 300 ng, and 500 ng of standard solutions and scanning the chromatograms on three different days.

Table 11: Track Assignment for **Precision**

Track Assignment

Track	Vial ID	Description	Volume	Type
1	Oroxylum indicum extract	Oroxylum indicum extract	2.0 µL	Sample
2	Baicalein marker	Baicalein marker	2.0 µL	Reference
3	Oroxylum indicum extract	Oroxylum indicum extract	4.0 µL	Sample

Track Assignment notes

Specificity

Different spots were demonstrated Specificity was by analyzing *Oroxylum indicum*(L.) kurz root extract (1000 µg/ml), standard solutions (100 µg/ml), mobile phase, and diluent in this parameter. However, only the standard and sample had RF values recorded, whereas the diluent and mobile phase did not have any spots. Spotting was performed and suggests that the technique is unique to the measurement of baicalein at specific volumes and plates were scanned at 254 nm and 366 nm using a CAMAG HPTLC Scanner V.

Table 12: Track Assignment for **Specificity**

Track Assignment

Track	Vial ID	Description	Volume	Type
1	Baicalein marker	Baicalein marker	2.0 µL	Reference
2	Baicalein marker	Baicalein marker	2.0 µL	Reference
3	Mobile phase	Mobile phase	2.0 µL	Reference
4	Mobile phase	Mobile phase	2.0 µL	Reference
5	Diluent	Diluent	2.0 µL	Reference
6	Diluent	Diluent	2.0 µL	Reference
7	Oroxylum indicum extract	Oroxylum indicum extract	2.0 µL	Sample
8	Oroxylum indicum extract	Oroxylum indicum extract	2.0 µL	Sample

Track Assignment notes

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

In terms of the limit of detection (LOD) and limit of quantification (LOQ), the sensitivity of the suggested approach for identifying quercetin, α-tocopherol, and β-carotene was assessed. The standard deviation (SD) and slope of the intercept were used to calculate these parameters. By using the average slope and the SD of the Intercept in the corresponding calculation methods, The LOD and LOQ values were determined.

LOD = 3.3 (σ/S)

LOQ = 10 (σ/S)

Where,

σ = represents the standard deviation of the y-intercepts from the regression lines. S = the slope of the calibration curve.

Table 13: Limit of detection and Limit of quantification

Drug	**LOD ng/spot**	**LOQ ng/spot**
Baicalein	0.908107127	2.751839802

Robustness

The robustness of the optimized HPTLC method was evaluated by implementing minor modifications in the mobile phase volume (±2 mL) and saturation time (±2 min). The results were expressed as a percentage of the Relative Standard Deviation (% RSD).

Table 14: Track Assignment for **Robustness**

Track Assignment

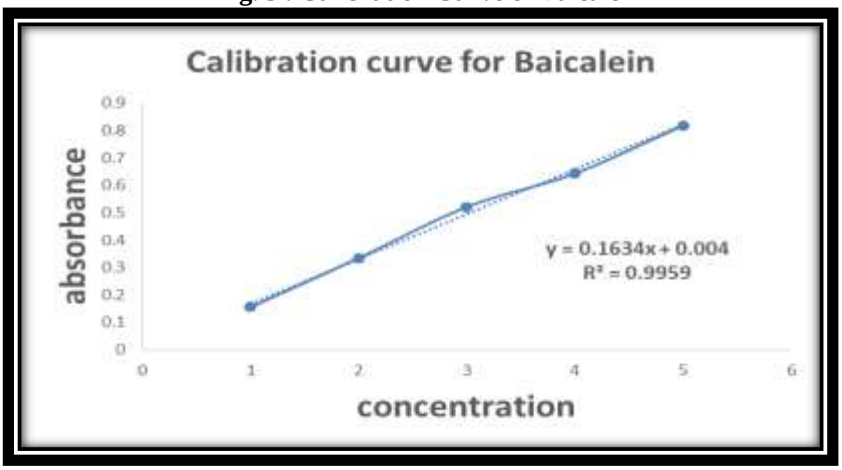
Track	Vial ID	Description	Volume	Type
1	Baicalein marker	Baicalein marker	5.0 µL	Reference
2	Oroxylum indicum extract	Oroxylum indicum extract	5.0 µL	Sample
3	Baicalein marker	Baicalein marker	5.0 µL	Reference
4	Oroxylum indicum extract	Oroxylum indicum extract	5.0 µL	Sample

Track Assignment notes

Quantification

Extracts from *Adenoon indicum* Dalz were administered in triplicate to pre-washed TLC plates using a sample applicator. The TLC plates were then prepared using an optimized mobile phase, as indicated by det *Adenoon indicum* Dalz in the preceding section. The peak areas for the marker were quantified, and the marker's concentration was determined by linear regression applied to the calibration curves.

Fig. 5 : Calibration Curve of Baicalein



Statistical analysis

All statistical data were calculated with Microsoft Excel.

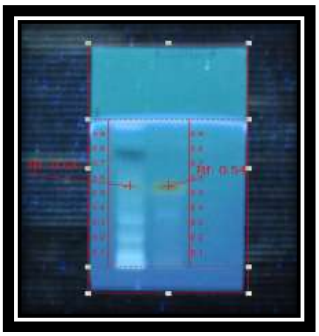
RESULT AND DISCUSSION

Detection and Visualization of Baicalein, Apigenin, Alloin, Proanthocyanidin, Betasitosterol, Gallic Acid, Quercetin, Lupiol

The produced plates were examined in both UV light (254nm) and visible light (366nm).

Baicalein

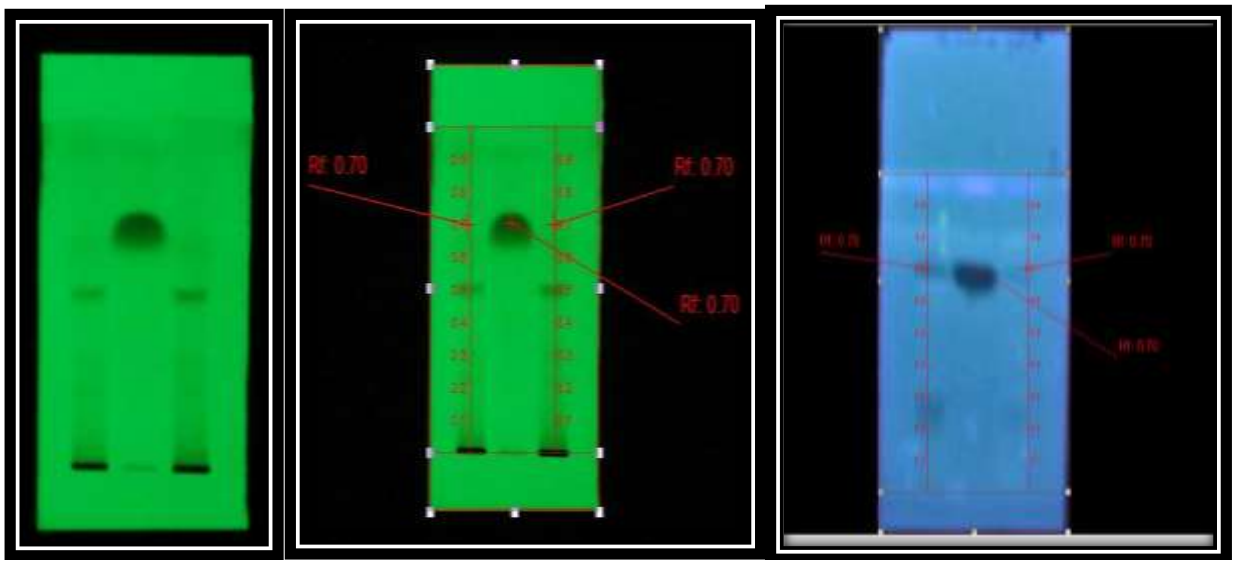
Fig. 6 : Rf Value of Baicalein recorded on HPTLC plate



This HPTLC plate developed using the mobile phase such as Toluene: Ethyl Acetate: Formic Acid (6:3:2, V/V/V) showed a Baicalein substance Rf value is 0.54

Alloin

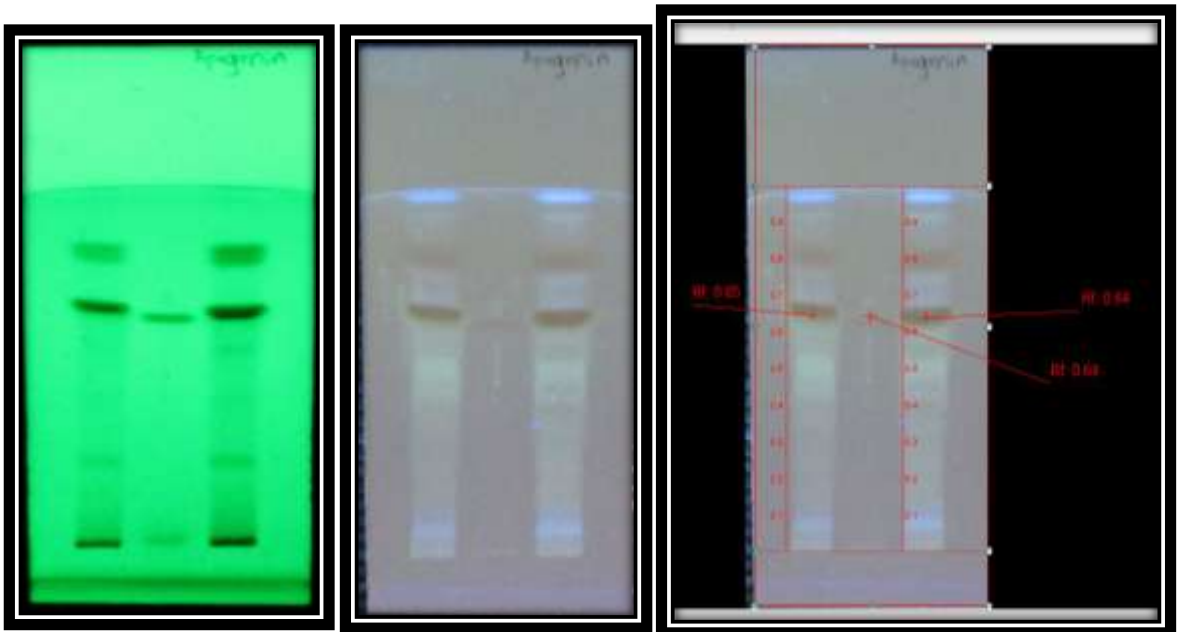
Fig. 7 : Rf Value Alloin recorded on HPTLC plate



This HPTLC plate developed using the mobile phase such as Chloroform: Ethyl acetate (7.5:2.5 ml) (V/V) showed Aloin substance Rf value is 0.70

Apigenin

Fig. 8 : Rf Value Apigenin recorded on HPTLC plate

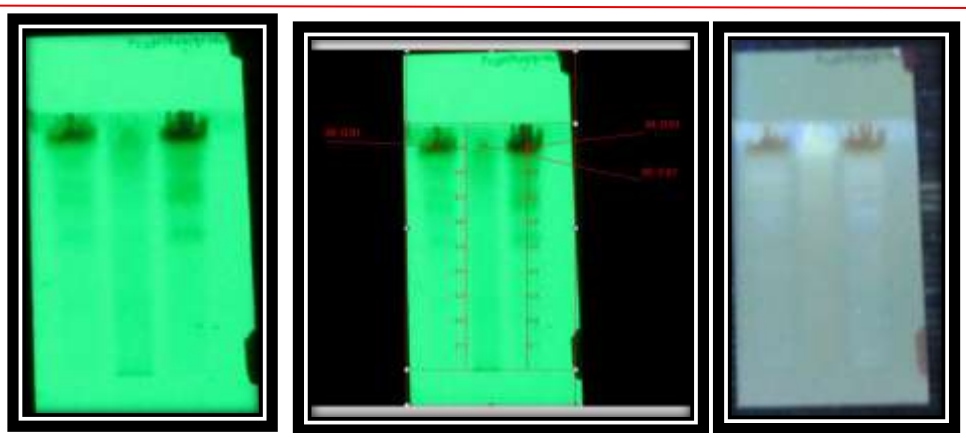


This HPTLC plate developed using the mobile phase such as Toluene: Ethyl acetate: Formic acid (6:4:0.2 ml) (V/V/V) showed a Apigenin substance Rf value is 0.64

Proanthocyanidine

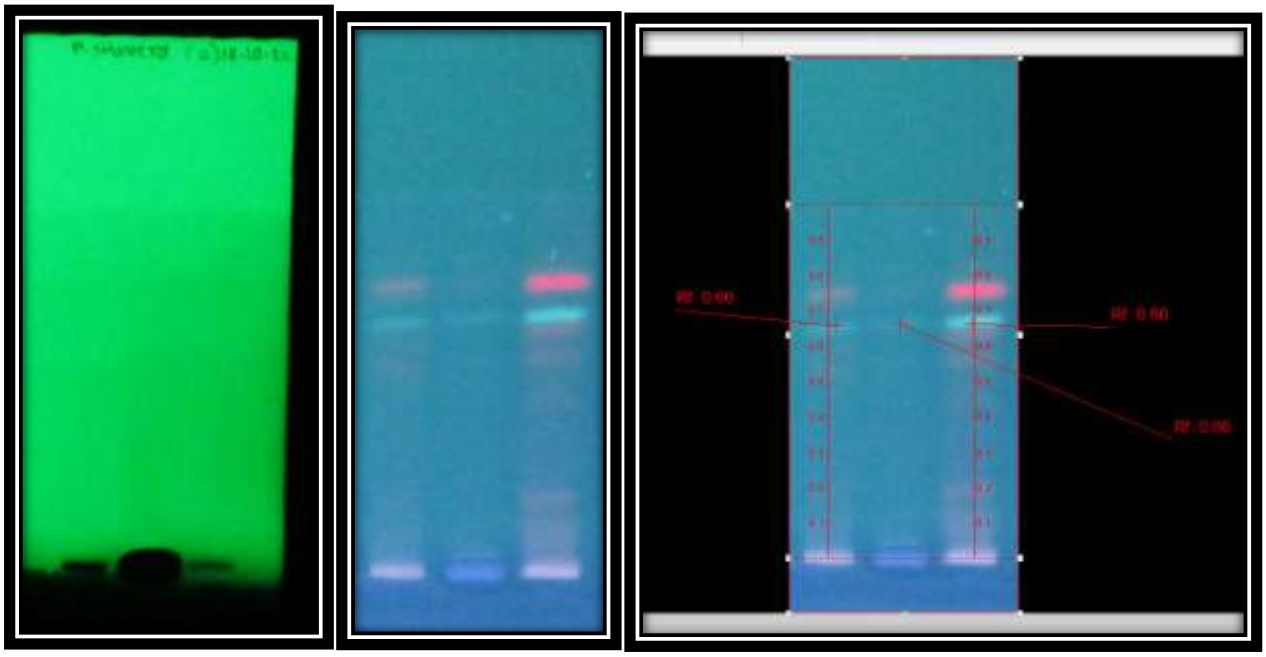
Fig. 9 : Rf Value Proanthocyanidine recorded on HPTLC plate

This HPTLC plate developed using the mobile phase such as Toluene: Ethyl acetate: Formic acid (7:2:1 ml) (V/V/V) showed a proanthocyanidin substance Rf value is 0.91



Betasitosterol

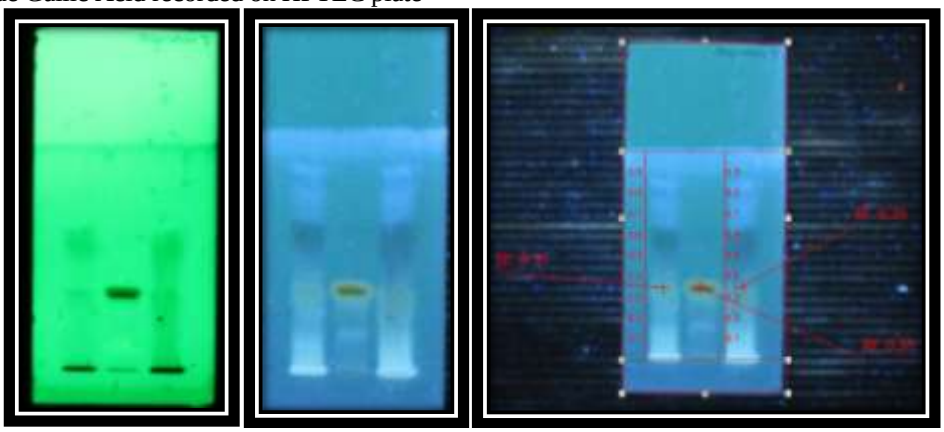
Fig. 10 : Rf Value Betasitosterol recorded on HPTLC plate



This HPTLC plate developed using the mobile phase such as Toluene: Ethyl acetate: Formic acid (7:3:1 ml) (V/V/V) showed a Betasitosterol substance Rf value is 0.66

Gallic Acid

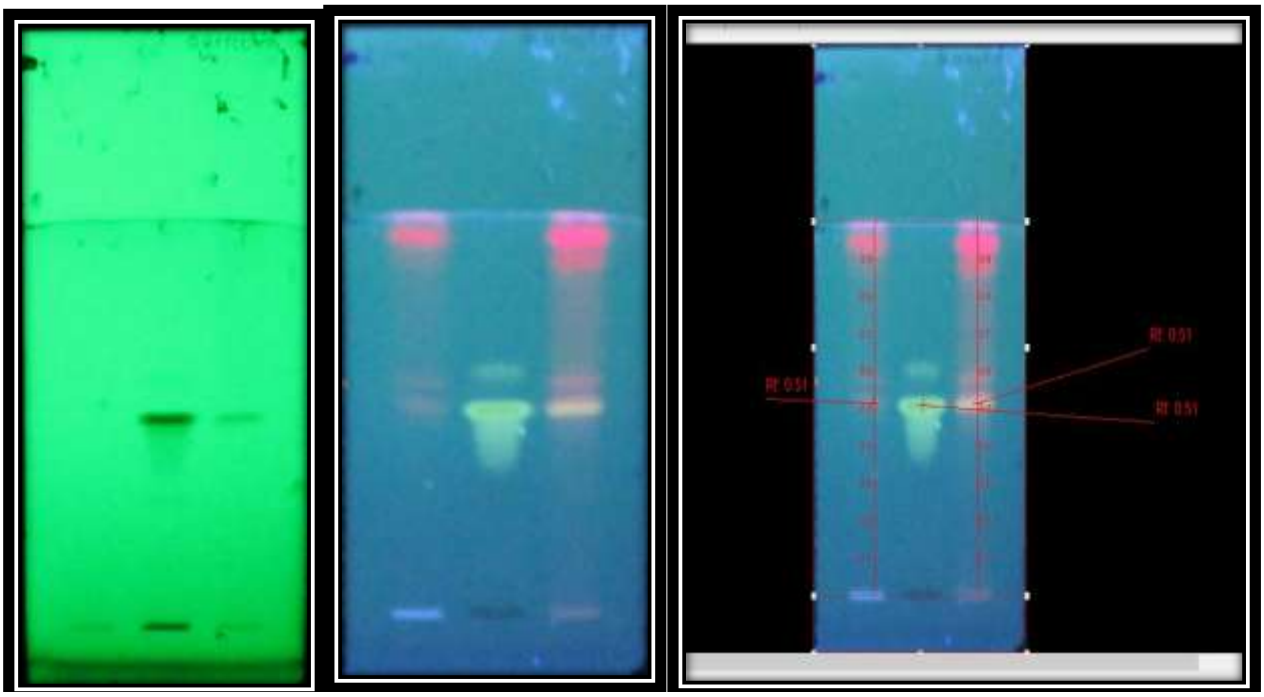
Fig. 11 : Rf Value Gallic Acid recorded on HPTLC plate



This HPTLC plate developed using the mobile phase such as Toluene: Ethyl acetate : Formic acid: Methanol (4:4:2:2 ml) (V/V/V) showed a Gallic Acid substance Rf value is 0.35

Quercetin

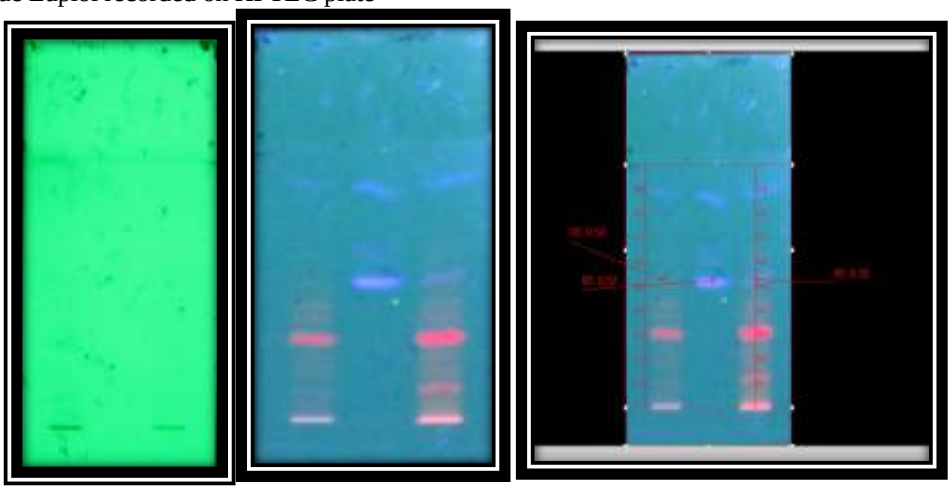
Fig. 12 : Rf Value Quercetin recorded on HPTLC plate



This HPTLC plate developed using the mobile phase such as Toluene: Ethyl acetate: Formic acid (5:4:1 ml) (V/V/V) showed a Quercetin substance Rf value is 0.51

Lupiol

Fig. 13 : Rf Value Lupiol recorded on HPTLC plate



This HPTLC plate developed using the mobile phase such as Glacial Acetic Acid: Methanol: Toluene (0.1:1:9 ml) (V/V/V) showed a Lupiol substance Rf value is 0.52

Method development

A glass CAMAG® Twin Trough Chamber (TTC) 10 × 10 cm was utilized to saturate the mobile phase for a duration of 20 min. Samples were deposited onto HPTLC plates utilizing a sample applicator equipped with a 100 µL microliter Syringe (ILS) and controlled nitrogen flow. The mobile phase, comprising Toluene: ethyl acetate: acetic acid in a ratio in a volumetric ratio of 6:3:2 (V/V/V), was freshly prepared and poured into a clean, dry twin trough chamber. The chamber was then allowed to saturate for 20 minutes before use. The plate was placed in a twin-trough critical glass chamber (CAMAG) pre-saturated with the mobile phase consisting of Toluene: ethyl acetate: acetic acid in a ratio in a volumetric ratio of 6:3:2 (V/V/V) and removed when the solvent reached up to 70 mm. After the development process, to recognize the bands, the plate was further dried by using hair dryer for 5 min and examined under UV light. The observed spots were sharp with no tailing, indicating a good separation and the R_f values for each band were recorded. The phytochemical components of *Oroxylum indicum*(L.) kurz root extract were separated by HPTLC analysis on pre-coated silica gel G 60 F254 HPTLC plates. After evaluating many solvent systems, a mobile phase consisting of Toluene: ethyl

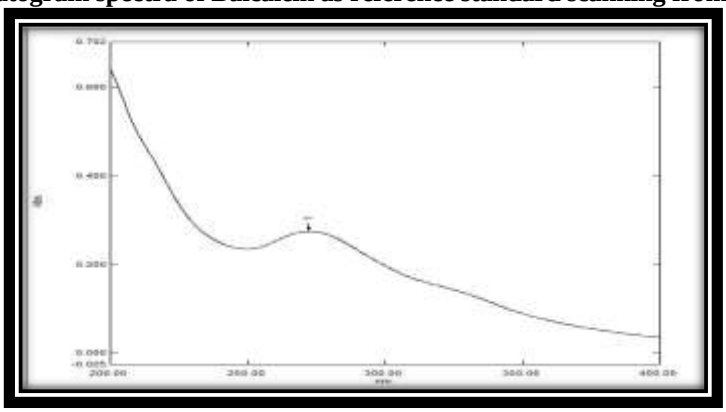
acetate: acetic acid in a ratio in a volumetric ratio of 6:3:2 (V/V/V) yielded the best separation. At 355 nm, the HPTLC plates showed discrete bands, indicating the presence of different phytochemicals (Figure 1). A key band with an R_f value of 0.54 suggested effective separation in all figures. A densitometric HPTLC method was devised to quantify Baicalein, with a UV spectrum overlay indicating peak purity at 355 nm, which corresponded to the Baicalein reference.

Method validation

The established HPTLC method effectively quantified Baicalein in *Oroxylum indicum(L.) kurz root extract*, matching important validation criteria outlined in the ICH standards. The calibration curves for Baicalein were linear between 100-500 ng/band, with a good correlation coefficient (r^2>0.9959), indicating the method's linearity (Table 1). Precision was strong, with % RSD values less than 2% for both repeatability and intermediate precision, indicating consistent results. Method robustness was maintained, as % RSD values remained below 5% despite slight changes in circumstances (Table 2), ensuring consistent performance across a variety of scenarios.

UV Spectroscopic Analysis:

Fig.14: Chromatogram spectra of Baicalein as reference standard scanning from 200 to 400 nm



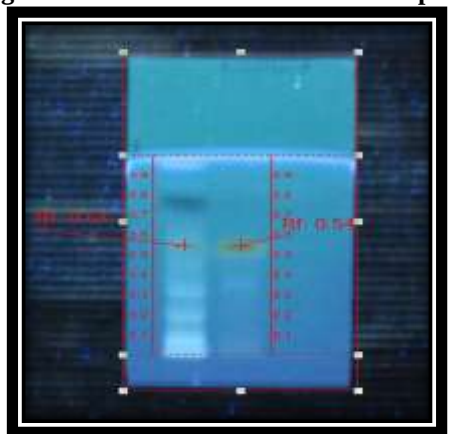
RF Value:

Under visible light, the generated chromatographic plate was analyzed.

Table 15: **RF Value**

Drug	Rf value
Baicalein	0.54

Fig. 15 : Rf Value recorded on HPTLC plate



Method application

Linearity

Fig. 16 : Calibration curve of Baicalein (Concentration v/s absorbance)

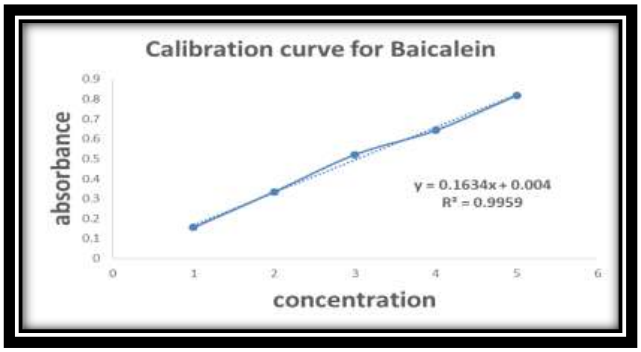


Table 7: Linearity validation results

Parameters	Baicalein
Detection	355
Linearity Range	100-500
Slope	0.016341
Intercept	0.020108987
Correlation Coefficient	0.9959
LOD	0.908107127
LOQ	2.751839802

Fig. 17 : Peak of standard Baicalein for Linearity

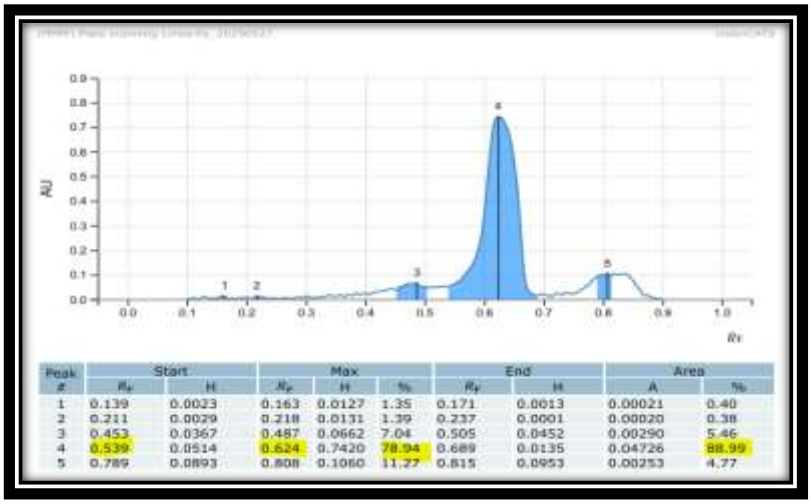


Fig. 18 : Peak for Linearity of standard Baicalein CAMAG TLC Scanner 4

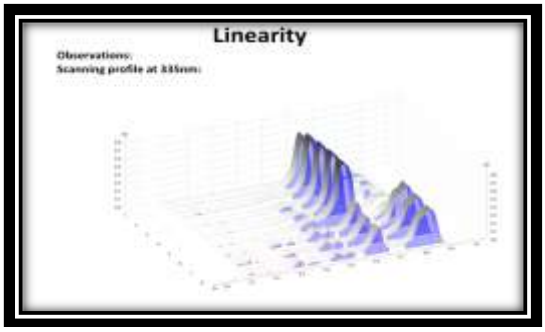


Fig. 19 : Linearity plate under 254 nm

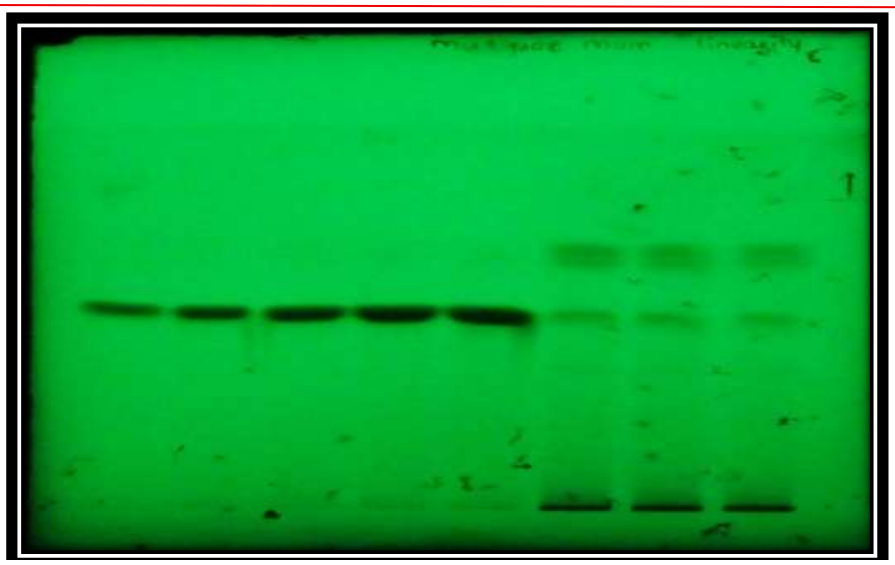
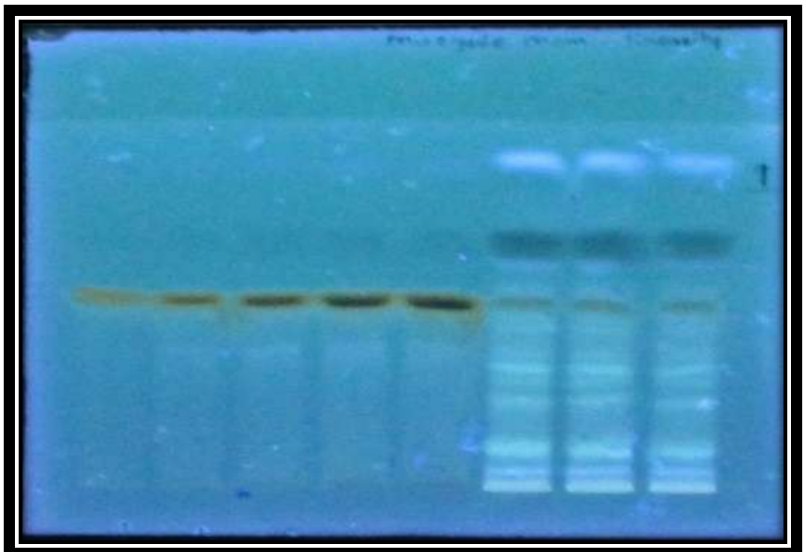


Fig. 20 : Linearity plate under 366 nm



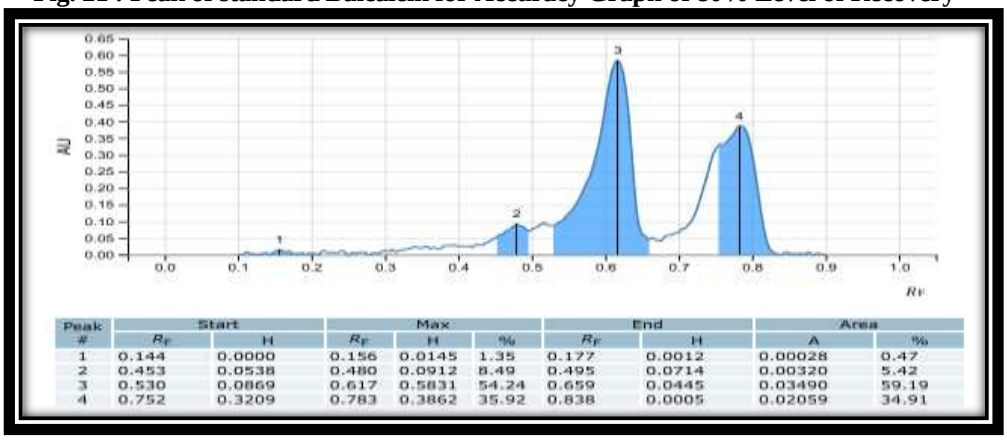
Accuracy

- % Recovery:
  - Baicalein = 78.54667 %
- Within acceptable limits, confirms accuracy.

Table 16 : Accuracy/ Recovery Validation Result

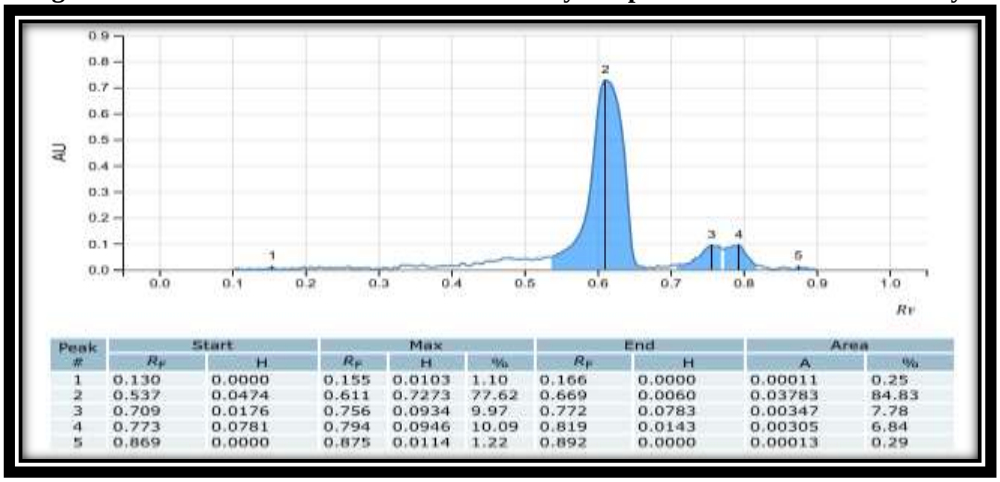
Analyte Name	**Baicalein**		
Level of recovery	80%	100%	120%
Mean Conc.	59.19	84.83	91.62
Percent Recovery	78.54667 %		

Fig. 21 : Peak of standard Baicalein for Accuracy Graph of 80% Level of Recovery



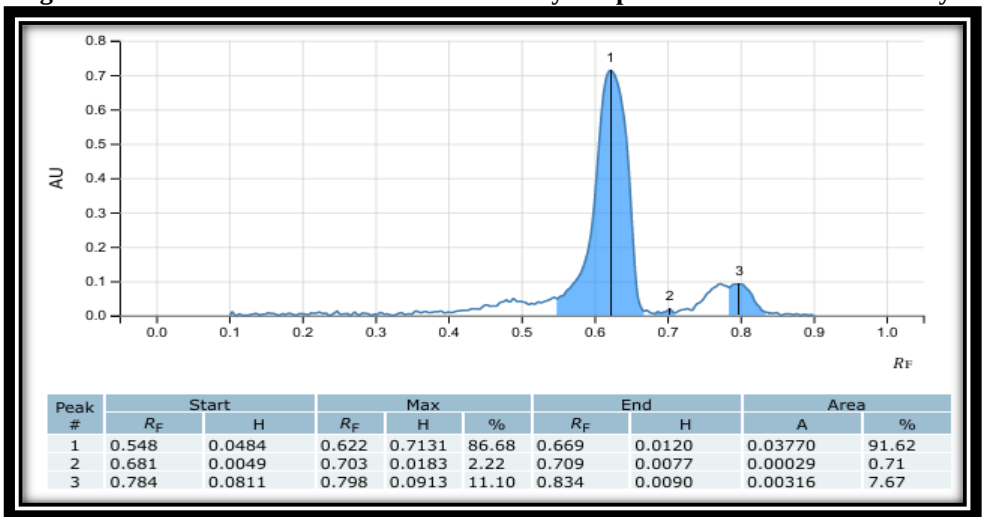
Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.144	0.0000	0.156	0.0145	1.35	0.177	0.0012	0.00028	0.47
2	0.453	0.0538	0.480	0.0912	8.49	0.495	0.0714	0.00320	5.42
3	0.530	0.0869	0.617	0.5831	54.24	0.659	0.0445	0.03490	59.19
4	0.752	0.3209	0.783	0.3862	35.92	0.838	0.0005	0.02059	34.91

Fig. 22 : Peak of standard Baicalein for Accuracy Graph of 100% Level of Recovery



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.130	0.0000	0.155	0.0103	1.10	0.166	0.0000	0.00011	0.25
2	0.537	0.0474	0.611	0.7273	77.62	0.669	0.0060	0.03783	84.83
3	0.709	0.0176	0.756	0.0934	9.97	0.772	0.0783	0.00347	7.78
4	0.773	0.0781	0.794	0.0946	10.09	0.819	0.0143	0.00305	6.84
5	0.869	0.0000	0.875	0.0114	1.22	0.892	0.0000	0.00013	0.29

Fig. 23 : Peak of standard Baicalein for Accuracy Graph of 120% Level of Recovery



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.548	0.0484	0.622	0.7131	86.68	0.669	0.0120	0.03770	91.62
2	0.681	0.0049	0.703	0.0183	2.22	0.709	0.0077	0.00029	0.71
3	0.784	0.0811	0.798	0.0913	11.10	0.834	0.0090	0.00316	7.67

Fig. 24 : Peak for Accuracy of standard Baicalein CAMAG TLC Scanner 4

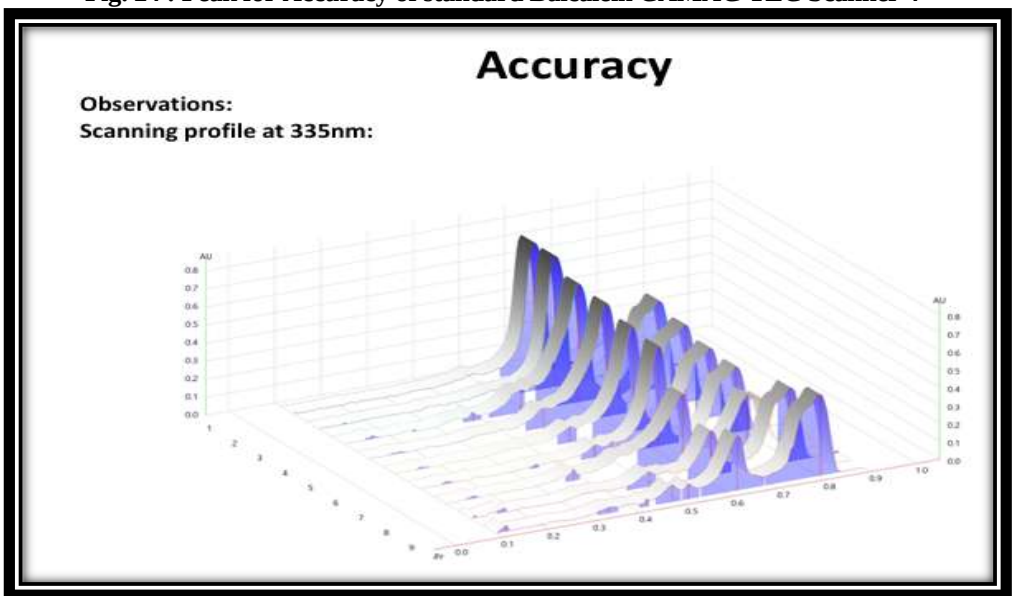


Fig. 25 : Accuracy plate under 254 nm

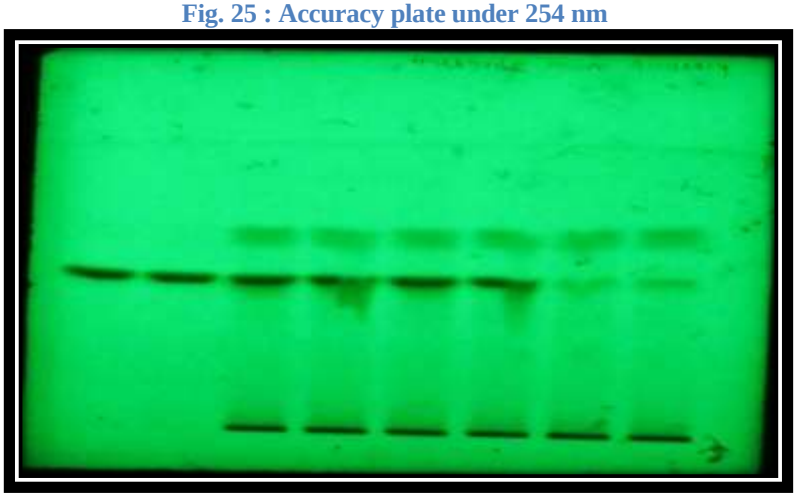
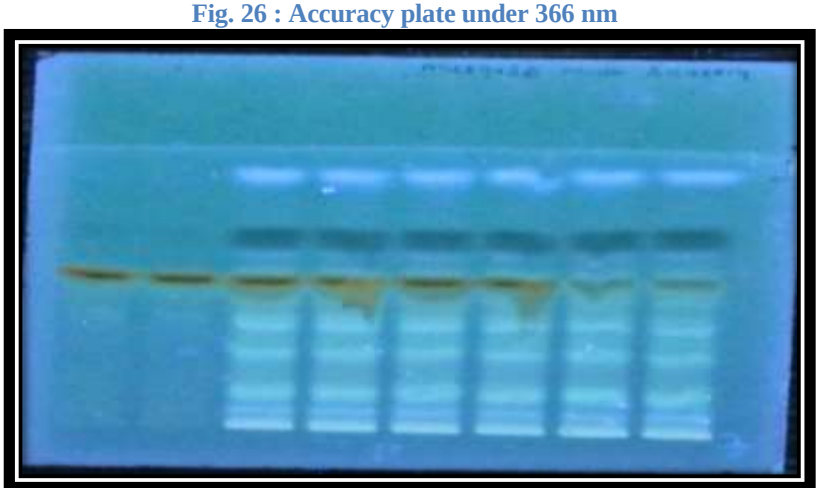
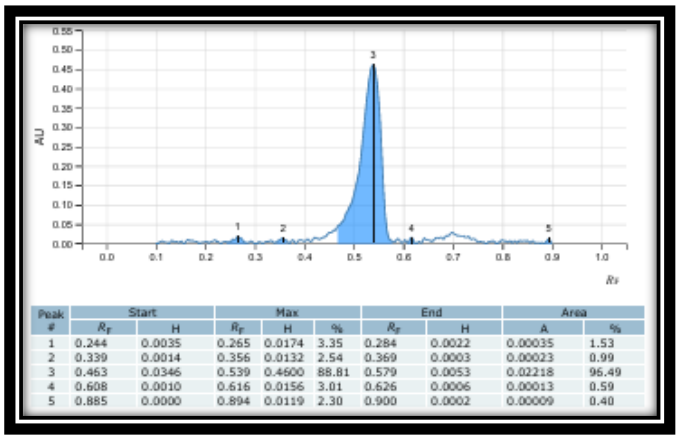


Fig. 26 : Accuracy plate under 366 nm



Precision

To assess the method's precision, the relative standard deviation (%RSD) was used. According to the tables below, all of the % RSD values derived from the repeatability research were less than 2.0%, proving that the method's precision is adequate.



Peak #	Start R_F	Start H	Max R_F	Max H	Max %	End R_F	End H	Area A	Area %
1	0.244	0.0035	0.265	0.0174	3.35	0.284	0.0022	0.00035	1.53
2	0.339	0.0014	0.356	0.0132	2.54	0.369	0.0003	0.00023	0.99
3	0.463	0.0346	0.539	0.4600	88.81	0.579	0.0053	0.02218	96.49
4	0.608	0.0010	0.616	0.0156	3.01	0.626	0.0006	0.00013	0.59
5	0.885	0.0000	0.894	0.0119	2.30	0.900	0.0002	0.00009	0.40

Fig. 28: Peak for precision of standard Baicalein CAMAG TLC Scanner 4

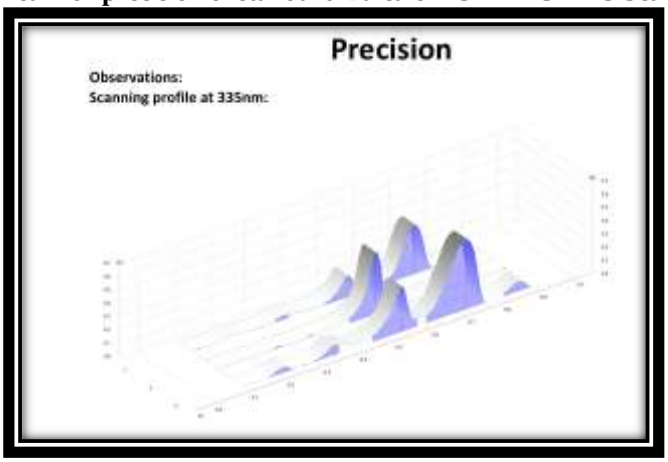


Fig. 29 : Precision plate under 254 nm

Fig. 30 : Precision plate under 366 nm with rf value 0.54

Table 17: Inter day precision statistical validation results

Concentration (ng/spot)	Day	Method	Area	Mean	Standard deviation	% RSD
200	1	Area	0.00007	**0.000813333**	**0.00105434**	**0.812479755**
	2	Area	0.00035			
	3	Area	0.00202			
400	1	Area	0.00786	**0.0111**	**0.009867**	**0.78047**
	2	Area	0.02218			
	3	Area	0.00326			

Fig. 30 : Precision plate under 366 nm with rf value 0.54

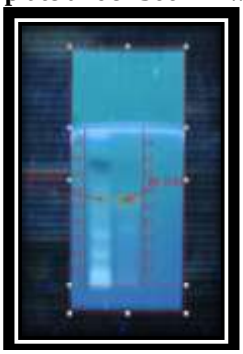


Fig. 31: Precision plate under 366 nm

Specificity

Peaks at Rf 0.54 observed in standard and extract.
No peaks for mobile phase/diluent ⇒ Method is specific.

Fig. 32: Peak of standard Baicalein for Specificity

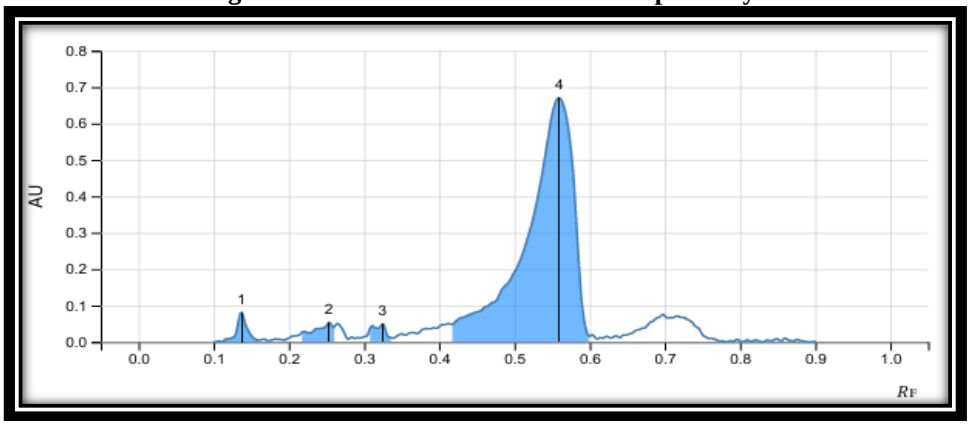


Fig. 33: Peak of Mobile phase for Specificity

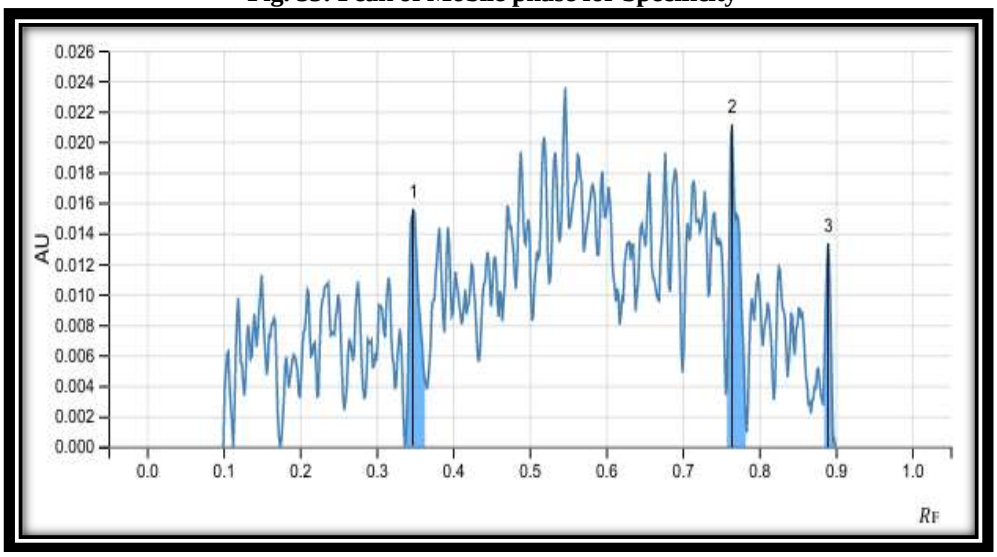


Fig. 34: Peak of Peak of Diluent for Specificity

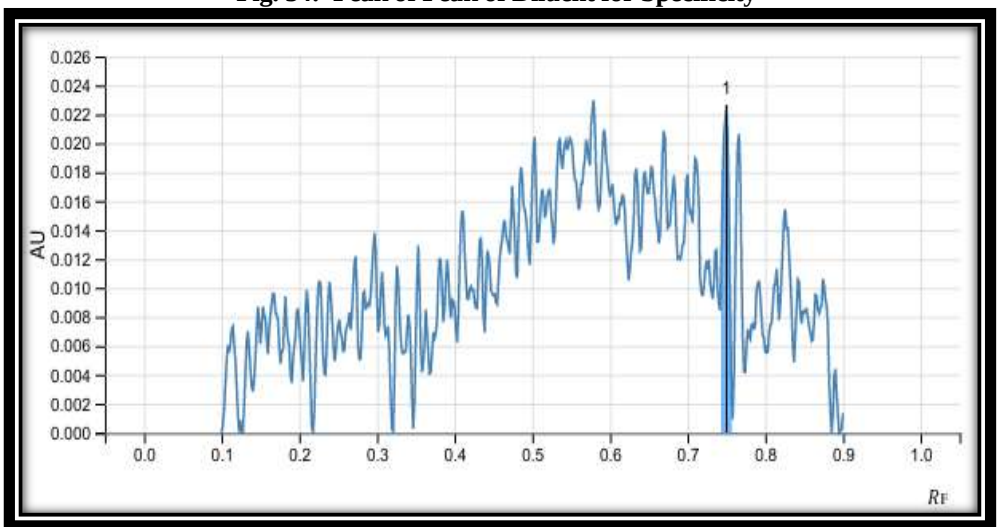


Fig. 35: Peak of *Oroxylum indicum*(L.) kurz root Extrat for Specificity

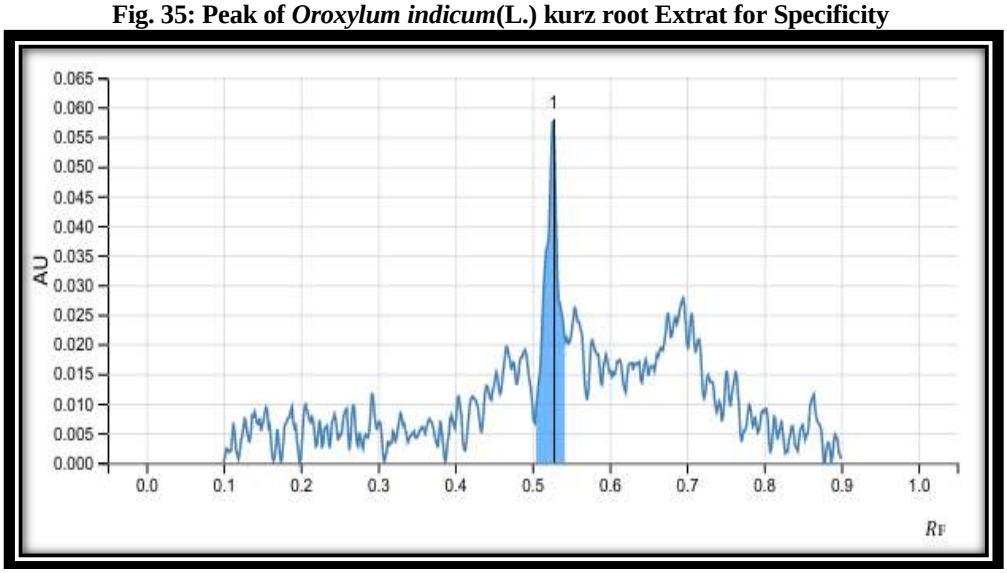


Fig. 36: Peak for Specificity of standard Baicalein CAMAG TLC Scanner 4

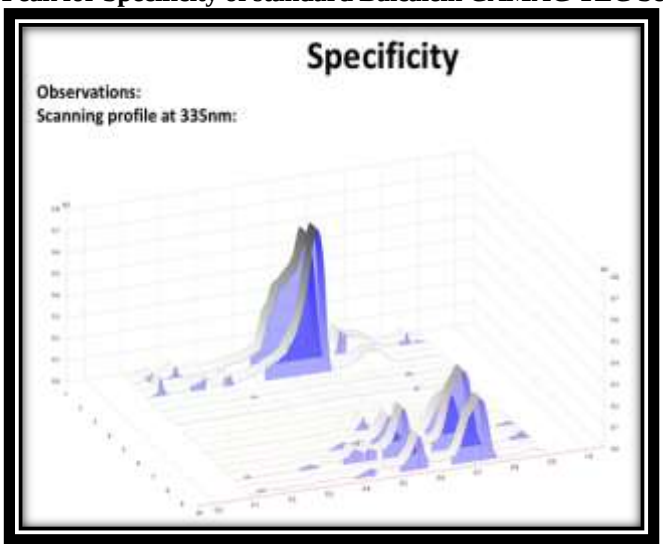


Fig. 37: Specificity plate under 254 nm

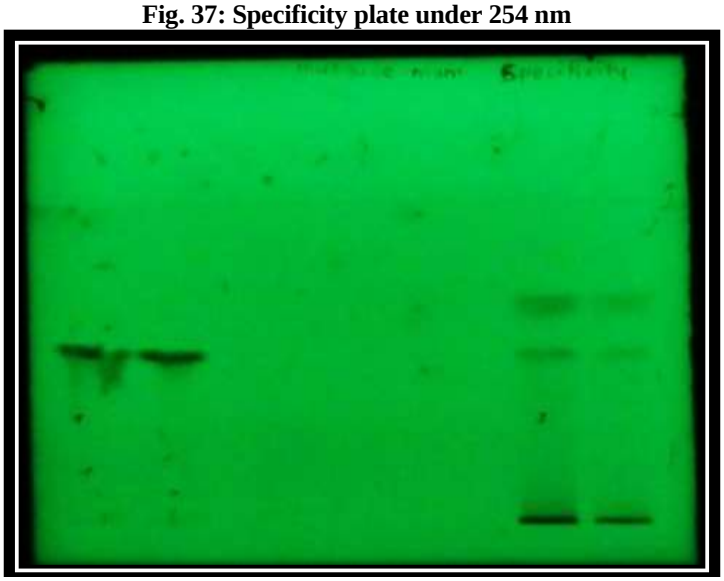
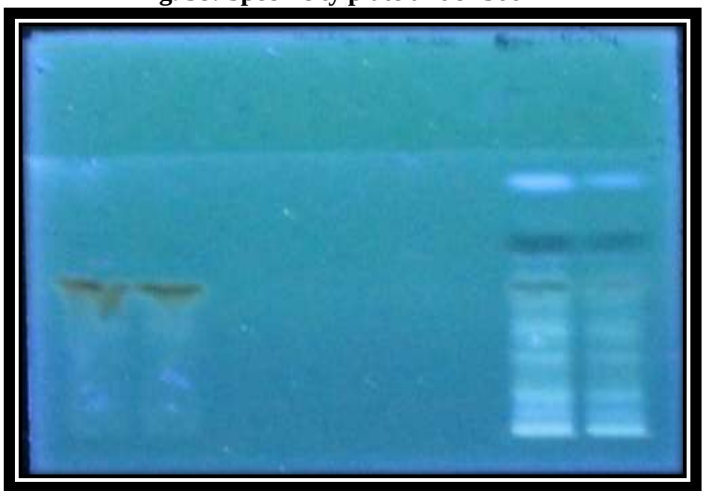


Fig. 38: Specificity plate under 366 nm



Limit of Detection (LOD) and Limit of Quantitation (LOQ)

In terms of the limit of detection (LOD) and limit of quantification (LOQ), the sensitivity of the suggested approach for identifying quercetin, α-tocopherol, and β-carotene was assessed. The standard deviation (SD) and slope of the intercept were used to calculate these parameters. By using the average slope and the SD of the Intercept in the corresponding calculation methods, The LOD and LOQ values were determined.

LOD = 3.3 (σ/S)
LOQ = 10 (σ/S)
Where,
σ = represents the standard deviation of the y-intercepts from the regression lines. S = the slope of the calibration curve.

Table 18: Limit of detection and quantification statistical validation results

Drug	LOD ng/spot	LOQ ng/spot
Baicalein	0.908107127	2.751839802

Robustness

Minor changes in saturation time showed %RSD < 0.5%, proving the method is robust.

Table 19: Robustness statistical validation results

Factor	Change	Peak area	SD	% RSD
Saturation time				
18	-10%	0.00825	0.019678	0.842139
20	0	0.05832	0.026394	0.933763
22	+10%	0.00732	0.003915	0.082956
mobile phase volume				

Fig. 39: Peak of standard Baicalein for Robustness

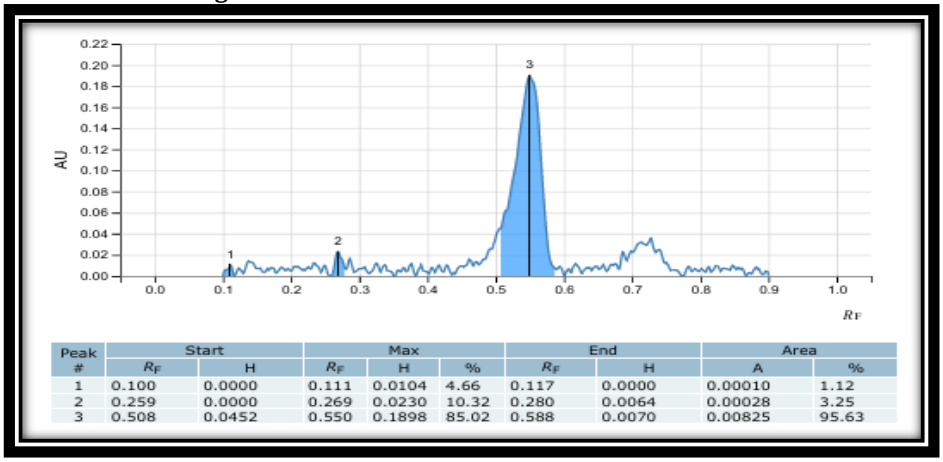


Fig. 40: Peak for Robustness of standard Baicalein CAMAG TLC Scanner 4

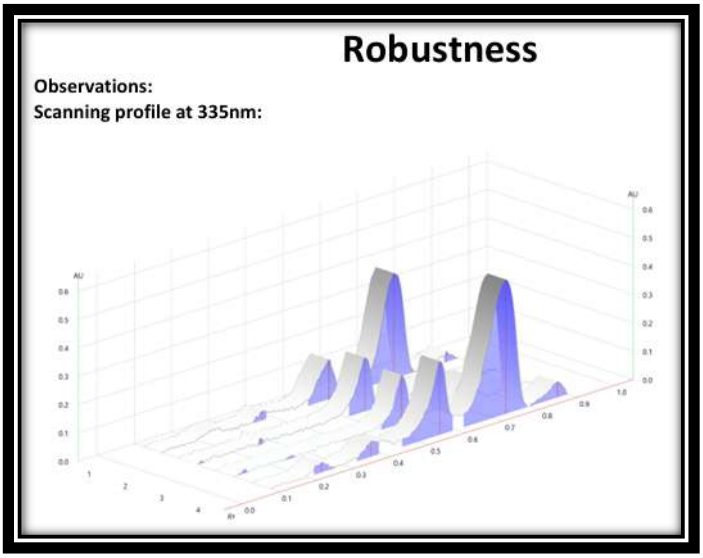


Fig. 41: Robustness plate under 254 nm

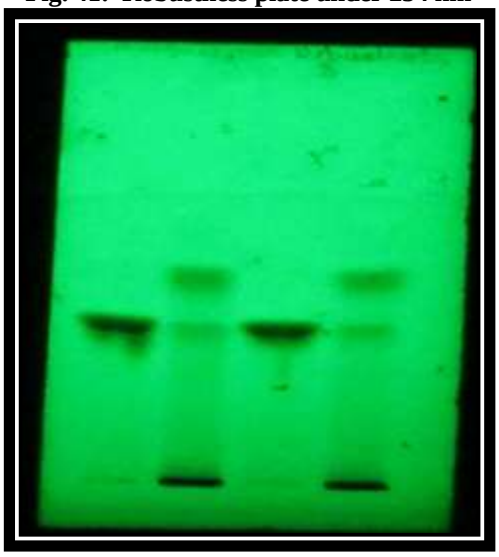
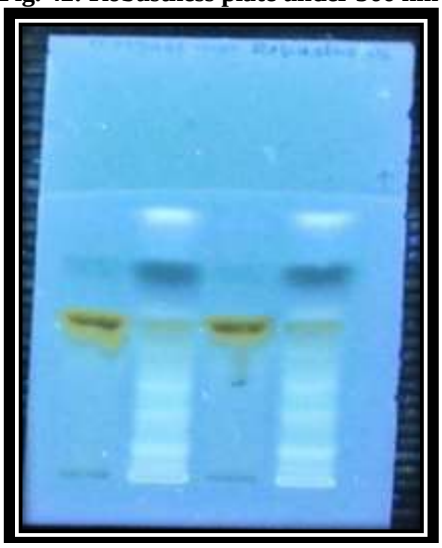


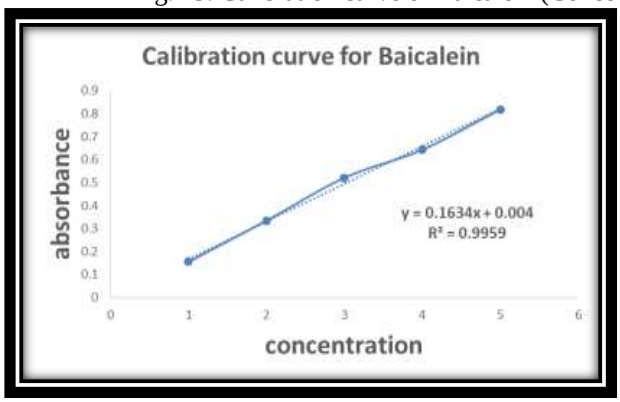
Fig. 42: Robustness plate under 366 nm



Quantification

Extracts from *Oroxylum indicum*(L.) kurz root were administered in triplicate to pre-washed TLC plates using a sample applicator. The TLC plates were then prepared using an optimized mobile phase, as indicated by *Oroxylum indicum*(L.) kurz root in the preceding section. The peak areas for the marker were quantified, and the marker's concentration was determined by linear regression applied to the calibration curves.

Fig. 43: Calibration curve of Baicalein (Concentration v/s absorbance)



X=concentration of baicalein in ng/mL, Y=Absorbance.

Statistical analysis

All statistical data were calculated with Microsoft Excel.

DISCUSSION

The scholars successfully HPTLC method optimized for separating and quantifying Baicalein content of *Oroxylum indicum*(L.) kurz root. The chosen solvent system Toluene: ethyl acetate: acetic acid in a ratio in a volumetric ratio of 6:3:2 (V/V/V) was successful in separating phytochemicals on silica gel plates. The R_F values and UV spectrum supported the method's specificity, resulting in precise Baicalein quantification without interference from other phytochemicals. Furthermore, the stability of the Baicalein

Table 20: Validation parameters by the proposed HPTLC method

Parameters	Baicalein
Range of linearity	**100-500 ng/band**
Regression equation	***y = 0.1634x + 0.004***
Correlation coefficient (R^2)	**R^2 = 0.9959**
Slope	**0.016341**
Intercept	**0.00401**
Repeatability (% RSD)	**0.49%-1.46%**
Intermediate precision (% RSD)	**0.36%-1.90%**
Recovery (%), (*n*=3)	**78.54667 %**
Limit of Detection (LOD)	**0.908107127**
Limit of Quantitation (LOQ)	**2.751839802**

This HPTLC method offers a consistent and specific methodology for assessing **Baicalein** in herbal extracts, making it a useful tool for quality control and phytochemical research.

The HPTLC method for **Baicalein** quantification in *Oroxylum indicum*(L.) kurz root extract was verified and shown to be extremely reliable, with strong linearity (r^2>0.995) across the concentration range, making it suitable for quantitative analysis. Low % RSD values imply good precision and reproducibility, but low LOD and LOQ values enable sensitive detection of tiny **Baicalein** levels. High recovery rates ensure an accurate portrayal of **Baicalein** content, making it suitable for quality control. Robustness testing revealed that the approach remains unaffected by slight alterations, making it appropriate for routine use. This dependable HPTLC approach improves the standardization and quality assessment of *Oroxylum indicum* (L.) kurz root extract products in herbal analysis.

CONCLUSION

A simple and sensitive HPTLC technique was successfully developed and validated a simple, accurate, and robust HPTLC method for detecting Baicalein content of Oroxylum indicum (L.) kurz root. Using silica gel 60 F_{254} plates and a mobile phase of Toluene: ethyl acetate: acetic acid in a ratio in a volumetric ratio of 6:3:2 (V/V/V), the method achieved clear separation with distinct Rf values. Validation as per ICH guidelines confirmed the method's linearity, specificity, accuracy, precision, and robustness. The findings support the standardization and quality control of Oroxylum indicum (L.) kurz root, highlighting its

The use of HPTLC to quantify **Baicalein** in *Oroxylum indicum* (L.) kurz root extracts has various advantages over other analytical approaches. Thin Layer Chromatography (TLC) techniques, such as HPTLC, are frequently used to analyze pharmaceuticals, botanical products, foods, environmental samples, and clinical specimens. TLC's benefits, such as its simplicity, dependability, and adaptability for high-throughput analysis, make it an excellent choice for fingerprint profiling and quantification of important marker molecules in herbal medicines. As a result, the proposed HPTLC approach is ideal for regular analysis, quality control, and standardization of *Oroxylum indicum* (L.) kurz root based raw materials and polyherbal formulations.
potential for pharmaceutical applications and as a reliable analytical tool for herbal formulations.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ICH: International Conference on Harmonization; r2: Regression Coefficient; HPTLC: High-Performance Thin Layer Chromatography; LOD: Limit of Detection;

LOQ: Limit of Quantification; σ: Standard Deviation; RSD: Relative Standard Deviation; µg/mg: Microgram per Milligram; µL: Microliter; ng: Nanogram; S: Slope; y = mx + c: Linearity Equation.

SUMMARY

In this present study, the quantitative estimation of Baicalein in Oroxylum indicum (L.) kurz extracts and herbal preparations by HPTLC densitometric analysis has not been reported. This easy, sensitive, and cost-effective HPTLC method standardizes Oroxylum indicum (L.) kurz extract in herbal formulations and goods to ensure quality control. The scientific data helps with chemical profiling, quality control, and regulatory compliance for Oroxylum indicum (L.) kurz and its products.

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