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HPTLC-BASED QUANTIFICATION OF ANTIOXIDANT MARKER COMPOUNDS IN *SAUSSUREA LAPPA* C.B. CLARKE AND THEIR THERAPEUTIC RELEVANCE IN KABASURA KUDINEER

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Keywords:

Saussurea lappa, HPTLC, Antioxidant markers, Herbal drug standardization, Kabasura Kudineer

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ABSTRACT: *Saussurea lappa* C.B. Clarke (Asteraceae), an important medicinal plant widely used in Ayurveda, Siddha, Unani, and Traditional Chinese Medicine, is a key ingredient of several classical formulations, including Kabasura Kudineer. Owing to its extensive therapeutic applications in inflammatory, respiratory, and infectious disorders, standardization and quality evaluation of this plant are essential. The present study aimed to estimate and quantify selected antioxidant marker compounds in *Saussurea lappa* roots using a High-Performance Thin Layer Chromatography (HPTLC) method. Methanolic extracts of authenticated *Saussurea lappa* root powder were analyzed alongside standard markers, including vitexin, gallic acid, mangiferin, kaempferol, rutin, quercetin, and rosmarinic acid. Chromatographic separation was achieved on silica gel 60 F254 plates using a mobile phase consisting of toluene: ethylacetate: formic acid: methanol (3:6:1.6:0.4 v/v/v/v). Densitometric scanning was performed at 254 nm and 366 nm. The method provided clear resolution with Rf values ranging from 0.08 to 0.91. Quantitative analysis revealed the presence of kaempferol (0.66%w/w), gallic acid (0.05%w/w), vitexin (0.02%w/w), and mangiferin (0.47%w/w) in *Saussurea lappa* extracts. This study provides a reliable HPTLC-based approach for phytochemical standardization and supports the role of *Saussurea lappa* in Siddha formulations, particularly Kabasura Kudineer.

INTRODUCTION: Herbal drugs show marked variation in their chemical composition owing to differences in source, cultivation, and processing, which necessitates the establishment of reliable analytical methods for quality control [1, 2].

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Although *Saussurea lappa* is widely used in traditional Siddha formulations such as Kabasura Kudineer, validated chromatographic methods for its marker-based standardization are scarce [3].

Therefore, a robust analytical approach is required to ensure identity, purity, and batch-to-batch consistency of the raw drug. The selected marker compounds rutin, gallic acid, quercetin, vitexin, mangiferin, rosmarinic acid, and kaempferol are representative polyphenolic and flavonoid constituents that are chemically stable, well-

resolved under chromatographic conditions, and suitable for quantitative analysis [4]. Their inclusion enables multi-marker profiling rather than dependence on a single constituent, thereby providing a comprehensive and reliable quality assessment. However, reports on the simultaneous estimation of these markers in *Saussurea lappa* using a single validated chromatographic method are limited [5, 6]. The present study addresses this gap by developing and validating a simple, precise, and reproducible analytical method for multi-marker quantification, which can be effectively applied for routine standardization and quality control of *Saussurea lappa* and its formulations [7, 8].

MATERIALS AND METHODS:

Collection of *Saussurea lappa* Raw Materials for HPTLC Screening: The root of *Saussurea lappa* Clarke (family: Asteraceae) was received as a gift sample from Traditional Siddha medical practitioner Mr. Cinnathambi, Boomidi, Dharmapuri District, Tamil Nadu and the plant material was authenticated based on macroscopic characteristics by Dr. S. Mutheeswaran, M.Sc., M.Phil., Ph.D., Scientist (Botany), Xavier Research Foundation, St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli – 627002, Tamil Nadu, India on 26 September 2025.A voucher specimen of the authenticated sample was deposited in the [Department of Pharmacognosy Herbarium, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India] under voucher specimen number [Voucher No.:SL/2025/26] for future reference. After authentication, the roots were coarsely powdered, and stored in airtight containers until further experimental use.

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 from Herbal Plant Formulation: The powdered root material of *Saussurea lappa* Clarke (10 g) was extracted with 100 mL of methanol (solvent-to-solid ratio 10:1, v/w), selected based on preliminary optimization studies and literature reports indicating efficient extraction of phenolic and flavonoid constituents using polar organic solvents. Standard stock solutions of rutin, gallic acid, quercetin, vitexin, mangiferin, rosmarinic acid, and kaempferol were prepared by accurately weighing 10 mg of each compound and transferring them individually into 10 mL volumetric flasks, followed by dissolution and volume adjustment with methanol. The extraction was carried out by sonication in an ultrasonic bath (40 kHz, 250 W) for 30 minutes at room temperature (25 ± 2 °C). After cooling, the extract was filtered through Whatman No. 1 filter paper and the filtrate was collected and estimated.

Application of Sample: Chromatographic analysis was performed on precoated aluminium-backed silica gel 60 F_{254} HPTLC plates (10 × 10 cm; Merck, Darmstadt, Germany).Sample application was carried out using a CAMAG Linomat V applicator equipped with a 100 µL Hamilton syringe under a continuous flow of nitrogen gas. A total of eight tracks were applied on plate. Samples were applied as bands of 6 mm width, positioned 10 mm from the lower edge and 10 mm from the side edge of the plate. The application rate was set at 150 nL s^{-1} to ensure uniform band formation and prevent sample diffusion.

Development: Chromatographic development was carried out in a CAMAG twin-trough glass development chamber (10 × 10 cm). Prior to development, the chamber was saturated with the mobile phase for 20 min at room temperature (25 ± 2 °C) using saturation filter paper lining the inner walls of the chamber to ensure uniform vapor equilibrium. The HPTLC plates were developed by the ascending technique to a migration distance of 80 mm from the point of sample application. After development, the plates were removed from the chamber and air-dried at room temperature to eliminate residual solvent prior to densitometric scanning.

Detection: Densitometric scanning was performed using a CAMAG TLC Scanner 3 controlled by winCATS software in absorbance–reflectance mode with a deuterium lamp as the radiation source, and the scanning speed was set at 20 mm/s. Chromatograms were initially recorded at 254 nm and 366 nm for peak detection and identity confirmation. Quantitative estimation was carried out at 254 nm for gallic acid, mangiferin, rutin,

quercetin, and kaempferol based on their maximum absorbance and higher signal intensity at this wavelength. Vitexin and rosmarinic acid were quantified at 366 nm, where these compounds exhibited greater sensitivity and improved peak resolution. Peak areas and corresponding Rf values were recorded and used for the calculation of marker compound content in *Saussurea lappa* root extracts.

RESULTS AND DISCUSSION: The following different solvent systems were evaluated for effective separation of phytoconstituents in *Saussurea lappa* root extract [9, 10], including 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 (93:7), and toluene:ethyl acetate:formic acid:methanol (3:6:1.6:0.4). Among these, the mobile phase toluene:ethyl acetate:formic acid:methanol (3:6:1.6:0.4, v/v/v/v/v) provided sharp, compact, and well-resolved bands for all selected marker compounds and was therefore selected for further analysis. Other solvent systems showed tailing, streaking, overlapping bands, or insufficient migration of polar constituents. The optimized chamber saturation time was 10 min 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, demonstrating adequate chromatographic resolution. Marker identification in the extract was confirmed by comparing Rf values and densitograms with those of corresponding reference standards. Quantitative analysis revealed the presence of kaempferol (0.66%), gallic acid (0.05%), vitexin (0.02%), and mangiferin (0.47%) in *Saussurea lappa* root extract **Table 1.** The results demonstrate that the developed HPTLC method enables simultaneous detection and quantification of multiple antioxidant marker compounds with good resolution and reproducibility. This multi-marker HPTLC approach provides a simple, rapid, and reliable method for phytochemical standardization and routine quality control of *Saussurea lappa* raw material and supports its consistent use in Siddha formulations such as Kabasura Kudineer. In Conclusion the antioxidant markers Kaempferol, gallic acid, vitexin, and Mangiferin were present in extracts. From the above findings that *Saussurea lappa* exerts its characteristic activity due to presence of antioxidant marker present in the extracts.

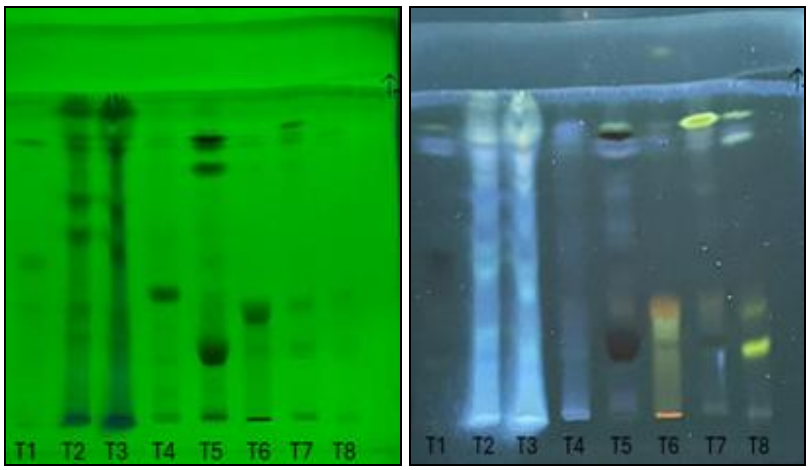

FIG. 1: HPTLC PROFILE OF *SAUSSUREA LAPPA* EXTRACT AND MARKERS AFTER DEVELOPMENT IN MOBILE PHASE AND VISUALISED AT 254 NM & 366 NM. T1 – *VITEXIN*, T2 – *SAUSSUREA LAPPA*, T3 – *SAUSSUREA LAPPA*, T4-ROSMARINIC ACID, T5-QUERCETIN, RUTIN, GALLIC ACID, T6-MANGIFERIN, T7-LUTEOLIN, T8-KAEMPFEROL

TABLE 1: RF VALUES OF STANDARD MARKERS IN EXTRACTS OF *SAUSSUREA LAPPA*

Track no.	Name / Amount of sample in µl	Rf values of marker in extracts	Name of the marker in extracts	Area of standard marker in sample	Amount of marker present in µg/ 5 µl & 10 µl of extracts / 5 µl of standards	Percent of marker present in sample
1	Vitexin (5µl)	0.42	Vitexin	58582.2	5.0	100%w/w

2	*Saussurea lappa (5µl)*	0.25	Kaempferol	8269.0	3.321	0.66%w/w
		0.75	Gallic acid	661.2	0.265	0.053%w/w
		0.40	Vitexin	1285.1	0.109	0.021%w/w
		0,85	Quercetin	3298.8	0.9273	0.09%w/w
3	*Saussurea lappa*	0.31	Mangiferin	10896.2	2.365	0.473%w/w
	(10µl)	0.86	Quercetin	1723.8	0.4853	0.09%w/w
4	Rosmarinic acid (5µl)	0.32	Rosmarinic acid	15939.3	5.0	100%w/w
5	Quercetin (5µl)	0.86	Quercetin	17758.7	5.0	100%w/w
	Rutin	0.19	Rutin	45259.9	5.0	100%w/w
	Gallic acid	0.77	Gallic acid	12453.6	5.0	100%w/w
6	Mangiferin (5µl)	0.32	Mangiferin	23029.2	5.0	100%w/w
7	Luteolin (5µl)	0.79	Luteolin	0.367.4	5.0	100%w/w
8	Kaempferol (5µl)	0.24	Kaempferol	5493.1	5.0	100%w/w

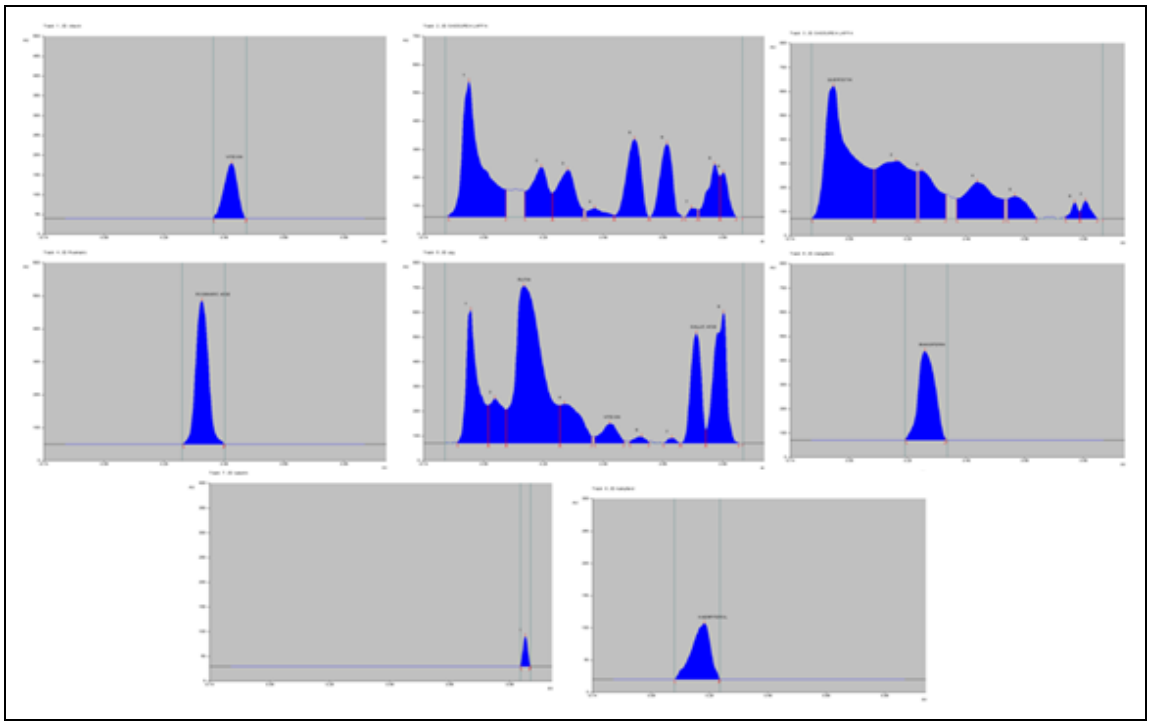

FIG. 2: CHROMATOGRAM OF *SAUSSUREA LAPPA* EXTRACTS AND STANDARD MARKERS. T1-VITEXIN, T2-*SAUSSUREA LAPPA*, T3-*SAUSSUREA LAPPA*, T4-ROSMARINIC ACID, T5-QUERCETIN, RUTIN, GALLIC ACID, T6-MANGIFERIN, T7-LUTEOLIN, T8-KAEMPFEROL

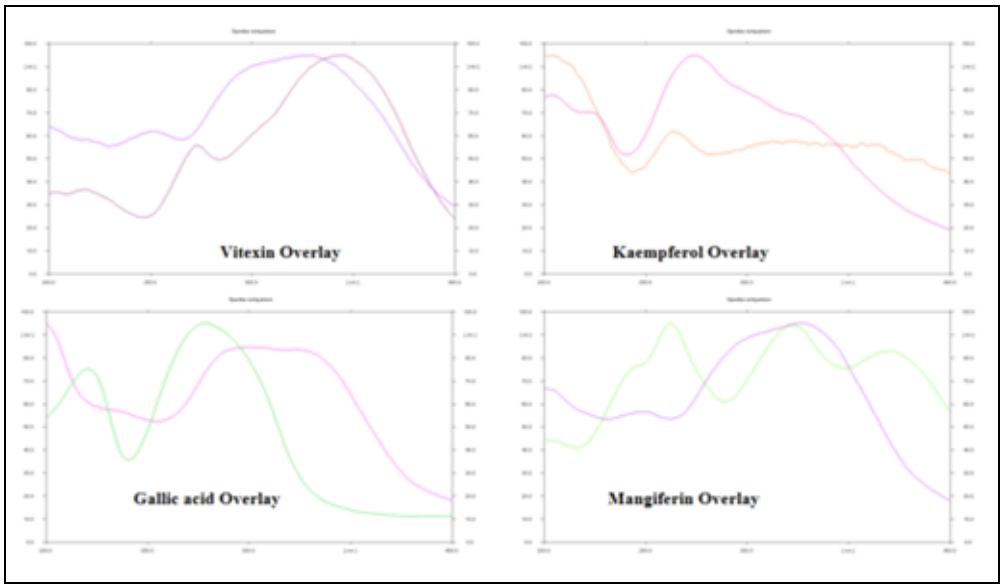

FIG. 3: OVERLAY OF VITEXIN, KAEMPFEROL, GALLIC ACID AND MANGIFERIN IN *SAUSSUREA LAPPA*

DISCUSSION: The present study was undertaken to develop and optimize an HPTLC method for the qualitative and quantitative determination of selected marker compounds in *Saussurea lappa* root extract. Several mobile phase systems with varying polarity were evaluated in order to achieve effective separation of phytoconstituents. Among the tested systems, toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4, v/v/v/v) provided sharp, well-resolved, and reproducible bands for all selected markers and was therefore chosen as the optimized mobile phase. Densitometric scanning at 254 nm enabled sensitive detection of the marker compounds with distinct Rf values, facilitating chromatographic fingerprinting of the extract. Marker identification was confirmed by comparison with reference standards and supported by co-spotting and spectral overlay, which improved the specificity and reliability of compound assignment. Quantitative analysis demonstrated the presence of kaempferol, gallic acid, vitexin, quercetin, and mangiferin in *Saussurea lappa* root extract at varying concentrations. Among these, kaempferol and mangiferin were observed in relatively higher amounts, indicating their predominance as phenolic constituents of the plant. Differences in marker content may be attributed to the chemical nature of the compounds and extraction efficiency. The reproducibility of Rf values and peak areas across replicate analyses confirmed the robustness and suitability of the optimized chromatographic conditions. The developed method allows simultaneous detection and quantification of multiple phytochemical markers within a single chromatographic run, making it efficient and cost-effective for routine analysis.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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