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Exploring the Coumarins from Stem Extract of *Ipomea Sagittiofolia Burm. f.* by High-Performance Thin Layer Chromatography

Raveesha Peeriga[1]*, S. A. Mohamed Shiek Arabath[2], Narayana Rao Alla[3], Ankireddy Yenireddy[4], Ganesh Kalla Venkata Bangaru[5]

[1]V. V. Institute of Pharmaceutical Sciences, Seshadri Rao Knowledge Village, Gudlavalleru - 521301, Krishna District, Andhra Pradesh, India.
[2]Arulmigu Kalasalingam College of Pharmacy, Krishnan Kovil-626126, Virudhunagar District, Tamilnadu, India.
[3]KL College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Andhra Pradesh, India.
[4]Narasaraopeta Engineering College, Palnadu District, Andhra Pradesh, India.
[5]Koneru Lakshmaiah University, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur District, Andhra Pradesh

Abstract

Coumarins are a class of secondary metabolites found in various plants, They have gained significant attention in the fields of Pharmacology and medicine due to their diverse biological activities and potential health benefits. High-Performance Thin-Layer Chromatography (HPTLC) plays a crucial role in the analysis and identification of secondary metabolites in plants. *Ipomoea sagittifolia*, commonly known as Arrow leaf Morning Glory or Heartleaf Morning Glory, is a species of flowering plant in the Convolvulaceae family. It is a climbing or trailing perennial vine native to tropical and subtropical regions of the Americas and some parts of Africa. *Ipomea sagittifolia* have some traditional uses and anecdotal evidence of its medicinal properties, further scientific research is needed to fully understand its potential health benefits and ensure its safety for use. Hence the current study is to explore the presence of coumarins in *Ipomea sagittifolia*. *Ipomea sagittifolia* stem is subjected for extraction by soxhlet apparatus. High Performance Thin Layer Chromatography analysis is performed for the Stem extract of Ipomea sagittofolia with derivatized agents. The results were shown peaks with stipulated Peak Height and Retardation Factor made a relevant proof for the presence of coumarins.

Keywords: Peak Height, Retardation factor, Coumarins, Convolvulacea

Full-length article **Corresponding Author*, e-mail: drprsha@gmail.com

1. Introduction

Coumarins are a class of natural compounds found in various plants, particularly in the Apiaceae (Umbelliferae) and Rutaceae families. They are aromatic compounds characterized by a benzene ring fused with an alpha-pyrone ring. Coumarins are known for their diverse biological activities and have been extensively studied for their pharmacological properties. These compounds have attracted significant attention in the scientific community due to their potential applications in medicine, agriculture, and food industries. Some of the notable properties of coumarins include anticoagulant, anti-inflammatory, antioxidant, antimicrobial, and anticancer activities [1-3]. The Convolvulaceae family, commonly known as the morning glory family, comprises a large group of flowering plants with more than 1,650 species. These plants are found in various habitats worldwide, including tropical and subtropical regions. Coumarins from the Convolvulaceae family may have potential medicinal, pharmacological, and ecological significance [4].

Ipomea sagittifolia is a species of flowering plant in the convolvulaceae family. It is commonly known as Arrow leaf Morning Glory. This plant is native to tropical and subtropical regions including Central America, South America, the Carribbean and some regions of Africa [5]. *Ipomea sagittifolia* is a climbing or trailing perennial vine with heart-shaped or arrowhead-shaped leaves hence the were called with common name Arrow leaf Morning Glory [6]. The leaves are glossy green. The flowers are typically funnel-shaped with varied shades of color viz., white, pink and lavender [7]. The plant produces attractive blooms that open in the morning. In some regions, *Ipomea sagittofolia* is used for medicinal purposes. Various parts of the plant such as leaves, stems and roots are believed to possess certain medicinal properties and are used in traditional herbal remedies [8-15]. Hence the current study is focused on

identify the coumarins from the stem extract of Ipomea sagittifolia belongs to the family convolvulaceae.

High-Performance Thin-Layer Chromatography (HPTLC) is a powerful and widely used chromatographic technique for the analysis and identification of secondary metabolites from plants [16-17]. It is a form of thin-layer chromatography (TLC) that offers enhanced performance, sensitivity, and efficiency in separating and analyzing complex mixtures of compounds found in plant extracts [18]. HPTLC plays a crucial role in phytochemical research, enabling the detection, quantification, and characterization of bioactive compounds, including alkaloids, flavonoids, terpenoids, phenolic compounds, and more, present in plant secondary metabolites [19]. HPTLC continues to be a valuable analytical tool in the field of phytochemistry, enabling researchers to explore and identify the diverse array of secondary metabolites present in plants, contributing to advancements in natural product research and drug discovery [20].

2. Materials and Methods

2.1. Extraction

The stems of the shrub *Ipomea sagittifolia* collected from Gudlavalleru's surrounds were clipped at the base. The stems were washed and given a week to shade dry. The stems were mechanically grounded after drying. Before being concentrated using a rotary evaporator, 100g of stalk powder was extracted with ethanol utilising a Soxhlet device.

2.2. Instrumentation

The ATS 4 applicator, TLC visualizer, and scanner 4 were all parts of the CAMAG HPTLC system utilized for the current study.

2.3. HPTLC Specifications

2.3.1. Preparation of Sample

1 mg of *Ipomea sagittifolia* stems was extracted with ethanol, concentrated in 1 ml of methanol, and filtered.

2.3.2. Development Chamber

After applying the sample, the HPTLC plate was kept in a development chamber containing mobile phase. Toluene, ethyl acetate, and methanol (4:4:1 v/v/v) were utilized in the mobile phase for steroid testing. 20 minutes pass before saturation.

2.3.3. Derivatization

The produced plate is derivatized for coumarins by adding 170 ml of methanol to a 200 ml glass bottle and cooling it in an ice-water bath. Mix thoroughly after adding 10 ml of sulfuric acid and 20 ml of acetic acid gradually. 1ml of anisaldehyde should be added after allowing the liquid to cool to room temperature [21-22].

2.3.4. Visualization

With the aid of TLC Scanner 4, the generated bands were successfully scanned at wavelengths of 366 nm and 540 nm.

3. Results and Discussion

The HPTLC analysis of the ethanolic stem extract of *Ipomea sagittifolia* revealed the presence of coumarins by examining the produced peaks in each chromatogram when scanned at 366nm and 540nm of 2.0µl and 5.0 µl each (Figs. 1 to 4). Hence further the plates were treated with derivatized reagent in order to enhance the detection and analysis of compounds. The chromatograms were acquired at wavelengths of 366nm and 540 nm of sample volume at 2.0µl and 5.0 µl (Track 1 & 2). The derivatized plates (Plate a, b and c) containing the samples were analysed of both volumes 2.0µl and 5.0 µl. The tables of chromatograms show the phytochemicals peak height and R_f values as well as their percent areas, and each chromatogram is described in the discussion part. *Ipomea sagittifolia's* ethanolic stem extract was subjected to HPTLC fingerprint analysis, which revealed the presence of coumarins. The generated band on the HPTLC plate examined around 366nm and 540nm are shown in Figure 1.

The chromatograms resulted for finger printing analysis of coumarins had shown well differentiated 8 peaks at R_f 0.008, 0.034, 0.276, 0.481, 0.597, 0.737, 0.897 and 0.968 at peak height 0.0675, 0.0162, 0.0309, 0.1916, 0.2076, 0.1681, 0.1024, 0.3034 at Sample Volume: 2.0µl (Track 1) and 11 peaks at shown R_f – 0.008, 0.034, 0.103, 0.256, 0.289, 0.473, 0.585, 0.731, 0.884, 0.924 and 0.953 at peak height 0.0906, 0.0268, 0.0278,. 0.0258, 0.0410, 0.2338, 0.3359, 0.2827, 0.2243, 0.2105 and 0.4316 at Sample Volume: 5.0µl (Track 2) at 540nm shown in Figure 2 &3. In derivatized plate b, the analysis shown that 7 peaks at R_f 0.011, 0.274, 0.482, 0.597, 0.756, 0.844, 0.968 at peak height 0.0304, 0.0268, 0.1003, 0.1235, 0.1047, 0.1110 and 0.1662 at Sample Volume: 2.0µl (Track 1) & shown 10 peaks at R_f 0.010, 0.102, 0.256, 0.289, 0.471, 0.585, 0.747, 0.827, 0.924 and 0.953 at peak height 0.0450, 0.0106, 0.0168, 0.0281, 0.1380, 0.2118, 0.2025, 0.2318, 0.1081 and 0.2602 at sample volume 5.0 µl (Track 2) at 366nm shown in Figure 4 & 5.

These Rf values demonstrated the presence of coumarins. Utilizing the Rf and peak values generated by HPTLC makes it easier to determine the kind and quantity of botanical components present in the plant. The separated compounds were easily visible on the HPTLC plates, which were made visible under UV of wavelengths 366 nm and 540 nm, respectively. Since they have been utilized for treating both acute and chronic ailments since the dawn of time, plant-derived phytoconstituents are essential for the production of medicines [23-24]. Due to the demand for plants, a quick analytical approach is necessary for the study and development of phytomedicines [25].

Applications for HPTLC include the investigation of phytochemicals and biological molecules, the measurement of medicines and active components, formulation fingerprinting, and the identification of adulterants in formulations. Using HPTLC, forensically significant chemicals can be discovered. In addition to other cutting-edge HPTLC-related methods, the employment of a hyphen in HPTLC-MS, HPTLC-FTIR, and HPTLC-Scanning Diode Laser has made HPTLC a potent analytical tool. Experts predict that the application of HPTLC to the investigation of medication formulations, bulk medicines, and natural materials will increase in the future [25].

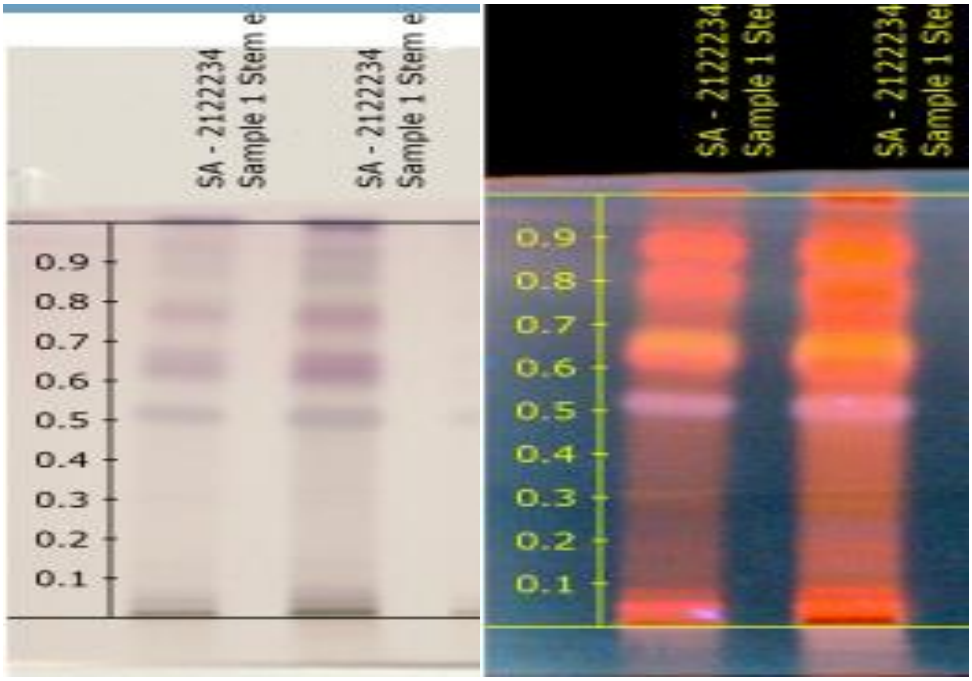
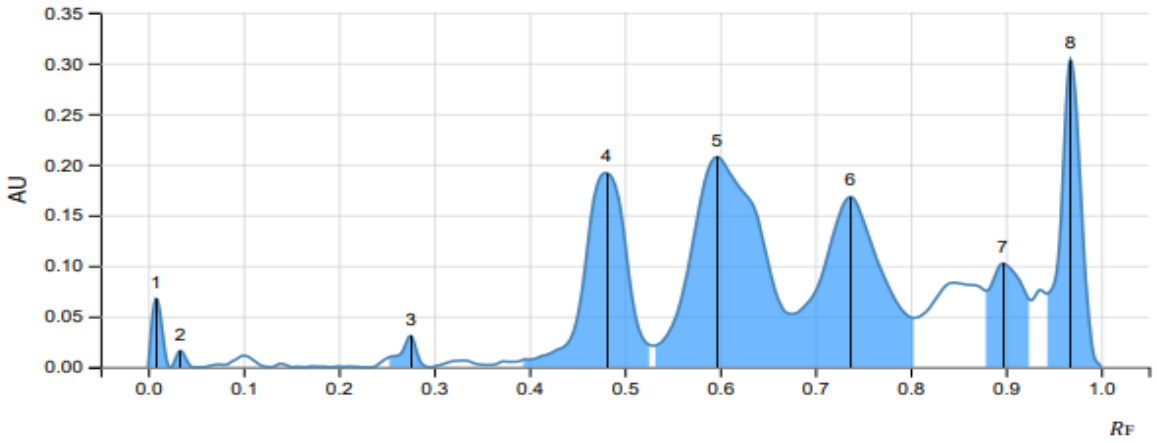
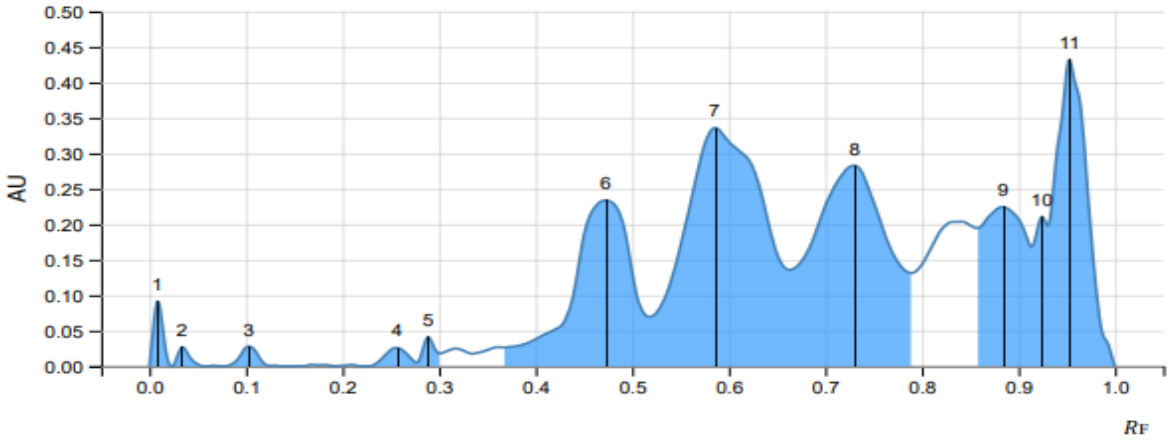


Figure 1: Derivatized Plates showing well-differentiated Bands at White & Remission



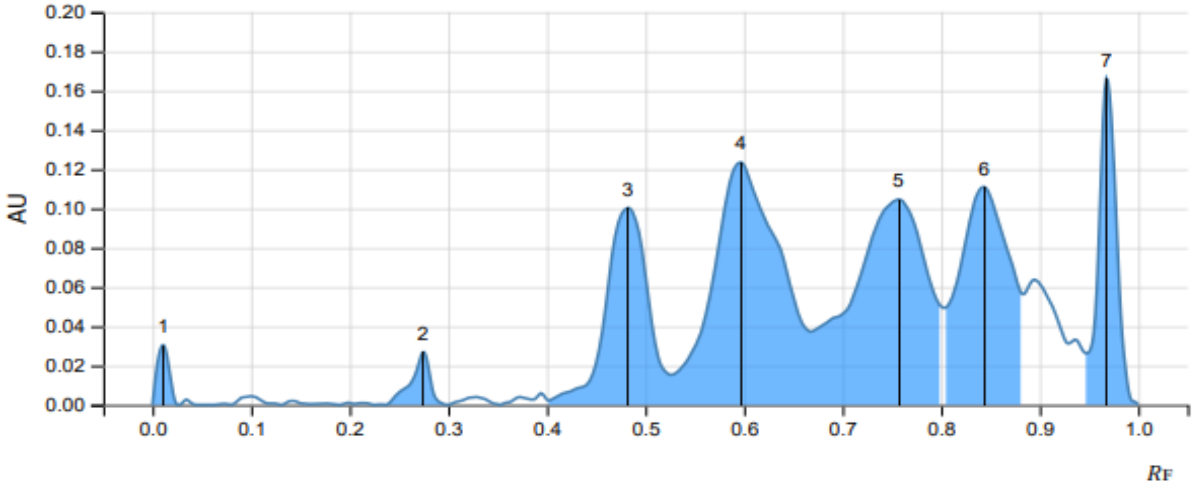
Peak #	Start		Max			End		Area		Manual peak	Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%		
1	0.000	0.0000	0.008	0.0675	6.21	0.023	0.0000	0.00082	1.54	No	
2	0.024	0.0000	0.034	0.0162	1.49	0.047	0.0000	0.00018	0.34	No	
3	0.252	0.0093	0.276	0.0309	2.84	0.295	0.0000	0.00059	1.11	No	
4	0.392	0.0068	0.481	0.1916	17.62	0.529	0.0212	0.00998	18.70	No	
5	0.531	0.0212	0.597	0.2076	19.09	0.674	0.0527	0.01740	32.57	No	
6	0.676	0.0526	0.737	0.1681	15.45	0.803	0.0485	0.01277	23.91	No	
7	0.879	0.0753	0.897	0.1024	9.41	0.926	0.0660	0.00411	7.70	No	
8	0.944	0.0723	0.968	0.3034	27.89	1.000	0.0002	0.00755	14.13	No	

Figure 2: HPTLC showing peak at 540nm at sample Volume 2.0µl after derivatization denotes presence of coumarins



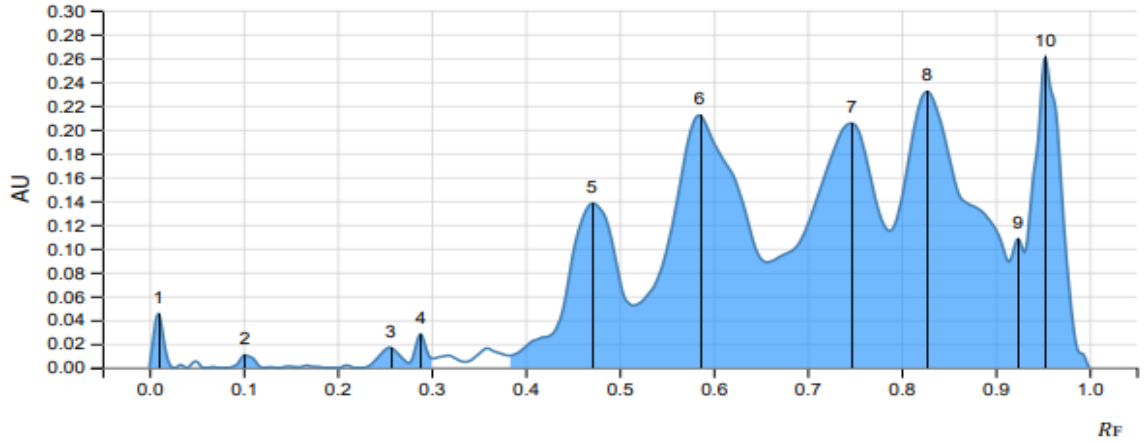
Peak #	Start		Max			End		Area		Manual peak	Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%		
1	0.000	0.0000	0.008	0.0906	4.69	0.023	0.0000	0.00102	0.93	No	
2	0.024	0.0000	0.034	0.0268	1.39	0.055	0.0000	0.00037	0.34	No	
3	0.079	0.0000	0.103	0.0278	1.44	0.126	0.0006	0.00057	0.52	No	
4	0.226	0.0000	0.256	0.0258	1.34	0.276	0.0052	0.00070	0.64	No	
5	0.276	0.0052	0.289	0.0410	2.12	0.302	0.0179	0.00062	0.57	No	
6	0.368	0.0265	0.473	0.2338	12.11	0.518	0.0696	0.01688	15.39	No	
7	0.518	0.0696	0.585	0.3359	17.40	0.663	0.1359	0.03251	29.65	No	
8	0.663	0.1359	0.731	0.2827	14.64	0.789	0.1312	0.02588	23.60	No	
9	0.858	0.1946	0.884	0.2243	11.62	0.913	0.1684	0.01141	10.40	No	
10	0.913	0.1684	0.924	0.2105	10.90	0.931	0.1985	0.00344	3.13	No	
11	0.931	0.1985	0.953	0.4316	22.35	1.000	0.0000	0.01626	14.83	No	

Figure 3: HPTLC showing peak at 540 nm at sample Volume 5.0µl after derivatization denotes presence of coumarins



Peak #	Start		Max			End		Area		Manual peak	Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%		
1	0.000	0.0000	0.011	0.0304	4.59	0.026	0.0000	0.00043	1.22	No	
2	0.237	0.0000	0.274	0.0268	4.04	0.298	0.0000	0.00058	1.65	No	
3	0.402	0.0022	0.482	0.1003	15.14	0.526	0.0152	0.00499	14.14	No	
4	0.526	0.0152	0.597	0.1235	18.64	0.668	0.0372	0.00987	27.96	No	
5	0.668	0.0372	0.756	0.1047	15.79	0.802	0.0495	0.00940	26.62	No	
6	0.803	0.0493	0.844	0.1110	16.74	0.882	0.0565	0.00653	18.50	No	
7	0.947	0.0262	0.968	0.1662	25.07	1.000	0.0002	0.00349	9.90	No	

Figure 4: HPTLC showing a peak at 366 nm at sample Volume 2.0µl after derivatization denotes the presence of coumarins



Peak #	Start		Max			End		Area		Manual peak	Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%		
1	0.000	0.0000	0.010	0.0450	3.58	0.026	0.0000	0.00053	0.68	No	
2	0.084	0.0000	0.102	0.0106	0.84	0.121	0.0000	0.00018	0.23	No	
3	0.227	0.0000	0.256	0.0168	1.34	0.276	0.0038	0.00043	0.55	No	
4	0.276	0.0038	0.289	0.0281	2.24	0.302	0.0075	0.00040	0.51	No	
5	0.382	0.0101	0.471	0.1380	10.99	0.515	0.0522	0.00885	11.25	No	
6	0.515	0.0522	0.585	0.2118	16.87	0.656	0.0884	0.01902	24.19	No	
7	0.656	0.0884	0.747	0.2052	16.35	0.787	0.1148	0.01867	23.73	No	
8	0.787	0.1148	0.827	0.2318	18.46	0.913	0.0898	0.02003	25.47	No	
9	0.915	0.0890	0.924	0.1081	8.61	0.931	0.0971	0.00162	2.06	No	
10	0.931	0.0971	0.953	0.2602	20.72	1.000	0.0000	0.00892	11.34	No	

Figure 5: HPTLC showing peak at 366 nm at sample Volume 5.0µl after derivatization denotes presence of coumarins

The primary goal of this research is to use HPTLC fingerprint analysis to identify botanical components including glycosides, essential oils, and tannins. Researchers use HPTLC to detect and analyze coumarins due to its ability to separate complex mixtures of compounds effectively [26-29]. The technique's sensitivity and reliability contribute to its widespread use in phytochemical research, allowing for the discovery and characterization of bioactive compounds, including coumarins, with potential health benefits and various pharmacological activities [30-32].

Ipomea sagittifolia leaves contain a range of coumrin types, according to the study's findings. The current study thus provides evidence that a variety of ailments can be successfully treated by conventional medicine. Numerous plants have secondary metabolites that are employed in the pharmaceutical, cosmetic, and pesticide industries. Steroids, alkaloids, flavanoids, saponins, terpenes, and a host of other chemicals are among these compounds. A crucial need for the use of these botanicals in research is the authenticity of medicinal plants in terms of both genetic and chemical properties. Taxonomy and the morphological characteristics of plants are useful in the systematic study of plants and their categorization in the era of molecular biology. The study's HPLTC profile's identification of coumarins is complemented, enhanced, and confirmed by the HPTLC profile (Chemical profile) of ethanolic extracts of *Ipomea sagittifolia*. The categorization of the plant according to chemotaxonomy is made easier with an awareness of the chemical elements that are present.

The presence of several coumarin types was detected by the varying number of peaks seen at 366 nm and 540 nm, respectively. The HPTLC profile of coumarins has shown the variance in biochemical levels that can occur. A linear, precise, and accurate HPTLC fingerprinting strategy may then be used to further characterize and validate the plant's therapeutic relevance.

The results of the current study are limited to the HPTLC analysis of an ethanolic extract of an *Ipomea sagittifolia* stem in order to ascertain the existence of various based on plants ingredients such as coumarins from chromatogram peaks and obtain peak tables. However, the research still necessitates quantifying these phytoconstituents.

4. Conclusions

According to the present study, the *Ipomea sagittifolia* plant apparently includes coumarins that are employed in traditional medicine to treat a number of ailments. For the production of medications, isolated components and their profile are essential. The HPTLC analysis for ethanol-based stem extract of *Ipomea sagittifolia* is useful in determining chemical profiles and bioactive components when the Rf values of these compounds are compared with standards as a reference.

References

[1] R.B. Teponno, S. Kusari, M. Spiteller. (2016). Recent advances in research on lignans and neolignans, Natural Product Reports. 33(8), 1044-1092.

[2] Y. Lu, R. Joerger, C. Wu. (2014). Study on extraction process of coumarin from Angelica archangelica Linn. Radix. Journal of Food Science and Technolology. 51(11), 3260-3267.

[3] M. Saleem, M. Nazir, M.S. Ali, H. Hussain, Y.S. Lee, N. Riaz. (2010). Antimicrobial natural products: an update on future antibiotic drug candidates, Natural Product Reports. 27(2), 238-254.

[4] Botanical Database Contributors. (2023). Convolvulaceae Family API. Plant Info Botany Database. https://www.plantinfobotanydatabase.com/api/convolvulaceae

[5] M. Figueiredo, T. Stevart, T.B. Toirambe. (2015). Inventory, use and management of convolvulacea species used by the local communities around the Bateke Plateau National Park, Democratic Republic of Congo. Ethnobotany Research and Applications. 14: 395-411.

[6] M.S. kumar, P.S. Deepa, M. Jayakumar, K. Kanna. (2018). Ethnomedicinal plants used by Iruler Tribe of Walajapet Taluk, Tamilnadu, India.

[7] Flora of China Editorial Committee. (2001). Flora of China (Vol. 16). Science Press, Beijing and Missouri Botanical Garden Press, St. Louis.

[8] R. Peeriga, A. Shaik, P. Kolli, A. Verooji, A. Kandula, P. Varma, B.B.R. Pathangi. (2022). Computational Study of Phytoconstituents in Myxopyrum Smilacifolium Blume against Inflammatory Mediator TNF-α. Journal of Drug and Alcohol Research. 11 (12): 1-5.

[9] R. Peeriga, K.P. Adarapu, K.S. Sanivarapu, J. Kanumuri, R.S. Akunuri, L.R. Atmakuri. (2022). Assessment of Anthelmintic Activity and in silico Study of Phytoconstituents in Decaschistia crotonifolia Wight & Arn. Root Extract. Journal of Young Pharmacists. 14(2), 169.

[10] R. Peeriga, K.P. Adarapu, A. Kurama, N. Mohammed, L.R. Atmakuri, D. Kumar. (2022). Insilico Investigation on Phytoconstituents in Pamburus missionis S. for Antioxidant Activity. Pharmacognosy Research. 14(3), 246-250.

[11] R. Peeriga, L.R. Atmakuri, S.S. Talluri, A. Tatineni, R.K. Tommandru, C.L. Varada, V.R. Gajula. (2023). Assessment of secondary metabolites in *Ipomea sagittifolia* leaf by hptlc fingerprints. Journal of Otolaryngology-Head and Neck Surgery. 27 (1): 1681-1691.

[12] R. Peeriga, B.S. Thelappilly, L.R. Atmakuri, A. Veeroji, M.S. Maragani, K.S.N. Venkata, R. Reddy, S. R.K. Yanamadala. (2023). Investigation of phenols and triterpenoids in *Ipomea sagittifolia* leaf by HPTLC fingerprints. Journal of Otolaryngology-Head and Neck Surgery. (1): 1672-1680.

[13] R. Peeriga, C.K. Bonnth. (2016). Pharmacognostical Investigation and Preliminary Phytochemical Screening of Leaves of Myxopyrum Smilacifolium B. Pharmacognosy Journal. 8(2), 159-164.

[14] K.B. Chandrasekhar, R. Peeriga. (2017). Evaluation of the antiarthritic activity of ethanolic leaf extract of Myxopyrum smilacifolium Blume. International Journal of Research in Medical Sciences. 3(10): 1-12.

[15] R. Peeriga, K.B. Chandrasekhar. Antiarthritic activity of leaf extracts of Pamburus missionis Swingle. (2017). International Journal of Pharm Science and Research. 8(2): 1-5.

[16] J. Sherma, B. Fried. (2003). Handbook of Thin-Layer Chromatography (3rd ed.). CRC Press. (ISBN: 978-0824707781).

[17] S. Erden. (2012). High-Performance thin-layer chromatography in phytochemical analysis. Journal of Pharmaceutical and Biomedical Analysis. 66: 11-20.

[18] E.S. Ong, S.F.Y. Li. (2004). High-Performance thin layer chromatography in the analysis of herbal medicines. Journal of Chromatography A. 1032 (1-2): 5-23.

[19] ICH Harmonised Tripartite Guideline. (2005). Validatjon for analytical procedures: Text and methodology Q2(R1). International Conference on Harmonization of Technical Requirements for Registration of Pharmaceutica for Human Use.

[20] S. Mohan, S. Hemalatha, M. Ayyanar. (2014). Thin layer chromatography: An effective tool for medicinal plant research. Journal of Medicinal Plants Studies. 2(6): 1-4.

[21] Authenticity of Essential Oils: A HPTLC Fingerprint Method (sigmaaldrich.com)

[22] A. Tseng. (1991). Chemoprevention of tumors in MTV-H ras transgenic mice with coumarins Proc. Am. Assoc. Cancer Research. 32: 2257.

[23] H. Liu. (2011) Extraction and isolation of compounds from herbal medicines. In: Willow, J and H. Liu (Eds.), Traditional Herbal Medicine Research Methods. John Wiley and Sons Inc.

[24] R. Bandameedi R.C.K. Babu. (2018). High-Performance Thin Layer Chromatography and Its Role Pharmaceutical Industry: Review. Journal of Bioscience and Bioengineering. 5 (3): 29-34.

[25] TLC Analysis of Digitalis Glycosides on HPTLC DIOL F254s application for TLC | Sigma-Aldrich (sigmaaldrich.com)

[26] S.B. Mahato, S. Sen. (1997). Advances in Coumarins Research. Research Signpost. ISBN: 978-8185669314

[27] S. Jokić, N. Radulović, N, R. Palić. (2011). Identification of coumarins in fruits of six Amorpha species by HPTLC. Journal of Planar Chromatography. 24(6): 479-482.

[28] Z. Maksimović, M. Jelikic-Stankov, M. Maksimović. (2004). Application of the HPTLC method for identification of coumarins in the extracts of five Pimpinella L. species. Journal of Planar Chromatography. 17(1): 39-42.

[29] D. Voinovich, G. Pizzimenti, R. Muzzalupo, A. Maggio. (2000). Identification and quantitative determination of coumarins in Seseli species by HPTLC. Journal of Planar Chromatography. 13(5). 325-330.

[30] J.C. Chalchat, M. Özcan, A. Michet. (1998). Direct identification of coumarins from different species of Pimpinella (Umbelliferae) by high-performance thin-layer chromatography. Journal of Chromatography A. 823(1-2): 295-301.

[31] R.P.P. Fernandes, M.G. Miguel, A.S. Lima. (2013). HPTLC and HPLC-DAD for the identification and quantification of coumarins in citrus fruits. Food Chemistry. 138(2-3): 1931-1936.