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Findings of Steroids, Coumarins and Saponins in *Ipomea sagittifolia* (Burn. f.)., Leaf Extract by HPTLC Fingerprints

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Abstract

Ipomea sagittifolia (Burn. f.)., was known with the common name "Morning glory", explored its usage from ancient times in Indian Traditional system of Medicine. Investigation of bioactive constituents using HPTLC scares up of phytoconstituents from an herb leads to the findings of about lead molecules having greater therapeutic importance. The Current work was to elucidate the presence of secondary metabolites like steroids, coumarins and saponins in methanolic leaf extract of *Ipomea sagittifolia* (Burn. f.)., by using CAMAG HPTLC System equipped with Linomat 5 Applicator, CAMAG ADC 2, TLC Visualizer with TLC Scanner 4. Analyzing the phytoconstituents by HPTLC densitometric analysis at 254nm and 366nm resulted in Chromatogram consisting of various peaks. The phytochemical profiling was carried out by interpretation of peaks, peak heights, peak area and R_f values obtained and represented in respective tables. The study revealed the presence of phytoconstituents steroids, coumarins and saponins which are accountable and to be explored for their therapeutic value which may excel for the treatment of various ailments.

Keywords: *Ipomea sagittifolia*, HPTLC, Morning Glory, Metabolite, Phytochemical Profiling

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

1. Introduction

Botanicals are the important biosources of medicines since ancient days. Integration of ethanobotany, medicine and plant natural product chemistry helps to achieve time saving and cost effective for finding out the active compounds [1]. India is rich in ethano-botanical Knowledge in other words it represents the Great tradition of Ayurveda, a living traditional practice even in this current era [2-3]. Standardization of botanicals is to be considered as the identity of natural materials may get affected due to evolution process and a correct authentication is needed to avoid adulteration in commercialization which is becoming a challenge now-a-days [4]. Hence it is very much needed to apply the typical modern analytical tools like HPTLC in order to standardize the botanicals as well as Ayurvedic [5-6]. *Ipomoea safittifolia (Burn. f.)* commonly known as Morning glory (in English), Laksmana (in Telugu), Manjikam (in Tamil), Asrabinducchada (in Sanskrit), bankalmi (in Hindi) belongs to the family Convolvulaceae. It is a climber consisting of simple leaves in alternate arrangement, ovate-heart shaped measures about 4-6cm long, 5-8 cm width. Flowers are funnel shaped bearing pink purple colored flower measures about 3-4cm long. In traditional system of medicine, it is used to promote strength, aphrodisiac, cures infertility in women and rejuvenates body cells and tissues [7]. To identify the main active components of medicinal plants, HPTLC makes sense for the extension of chromatographic fingerprints. In comparison to TLC, the separation and resolution are substantially superior, and the results are far more consistent and reproducible. It has the main benefit of in-situ qualitative and quantitative assessments using scanning densitometry when combined with automated digital scanning profiling [8]. Along with the vivid graphical representation, the HPTLC image also offers additional, understandable visible color and fluorescence

parameters for parallel evaluation on the same plate. Additionally, it demonstrated a more distinct separation of several secondary metabolites. HPTLC, an analytical technique is widely used in phytochemical profiling of natural products by many pharmaceutical industries for analyzing the lead molecules in drug discovery [9]. The current study was aimed for phytochemical profiling of *Ipomea saggitifolia*(ethanolic extract of leaf) by HPTLC technique.

2. Materials and Methods

2.1. Collection and Authentication

Leaves of *Ipomoea sagitttifolia* was collected in the month of November 2021, from surroundings of Gudivada namely Kauthavaram (Lat 16.3393388 & Long 81.0699462), Adada (Lat 16.3581262 & Long 80.9855956), Chitram (Lat 16.3387745 & Long 81.0309538) &Gudlavalleru (Lat 16.353774 & Long 81.041919), Andhra Pradesh. The plant material was identified by Prof. K. Madhava Shetty, Dept. of Botany, SV University, Tirupati, Andhra Pradesh, India.

2.2. Extraction

The leaves collected from the surroundings of Gudivada were collected by hand pricking, washed and shade dried for 7 days. The dried leaves were finely powdered by mechanical grinder. The powdered leaf material (100g) was extracted with methanol using hot percolation process by Soxhlet apparatus. Excess solvent was evaporated by rotary evaporator; crude extract was obtained and stored in airtight container. It was observed that the percentage yield of leaf extract from Gudlavalleru originated plant is higher when compared to other regions. Hence the leaf extract of the plant grown in Gudlavalleru region is preferred for chromatographical analysis.

2.3. Instrumentation

A CAMAG HPTLC system equipped with ATS 4 applicator, software LABSERVER, version 3.1.21109.3, TLC visualizer and TLC Scanner 4 was used.

2.4. Chemicals and solvents

The chromatography grade of chemicals and solvents were used for the study.

2.5. HPTLC Parameters

2.5.1. Preparation of test solution

100 mg of methanolic leaf extract of *Ipomea sagittifolia* was dissolved in 100ml methanol and filtered.

2.6. Chromatographic conditions

2.6.1. Sample Applicator

The HPTLC was performed on 100x100 mm pre-coated silica gel 60 F254 (MERCK) HPTLC Plate with solvent front position at 70mm. The sample was applied in bands on plate with the help of applicator ATS 4 at a rate of 150nl/s.

2.6.2. Development Chamber

Further the HPTLC plate (after sample application) was kept in development chamber comprising of TTC 10x10 with mobile phase Diethyl ether: Toulene (1:1), Toulene: Ethylacetate: Methanol (4:4:1) and Chloroform: Glacial acetic acid: Methanol: Water (64:32:12:8) allowing saturation for the period of 20 min with a volume front through 5ml, drying time was 5 min at room temperature for Coumarins, Steroids and Saponins.

2.7. Derivatization

For steroids, saponins, coumarins the extract is treated with Acetic anhydride and Sulphuric acid, Anisaldehyde Sulphuric acid reagent and 10 % Ethanolic KOH respectively.

2.8. Visualization

The developed band was visualized with the help of TLC visualizer, and it was scanned using TLC Scanner 4 at an optimized resolution, scanned speed of 20mm/s, at wavelength (s) 254nm (Absorbance, Lamp: Deuterium, Filter: K400), 366 nm (Fluorescence, Lamp: Mercury, Filter: K400) and 540 nm (Absorbance, Lamp: Tungsten, Filter: K400).

3. Results and Discussion

HPTLC analysis was performed on the methanolic leaf extract of *Ipomea sagittifolia* shown the presence class of compounds viz., steroids, coumarins and saponins. Figure 1 represents the developed bands on HPTLC plates scanned at 254nm and 366nm.The chromatogram resulted, scanned at 254 nm and 366nm were shown in Figure 2, 3 & Table 1, 2 for finger printing analysis of steroids had shown 1 peak (R_f Value: 0.969; Peak height (H): 0.0794) and 2 peaks (R_f Value: 0.098, 0.965; Peak height (H): 0.0794, 0.0935) in contrast, after derivatization had shown 4, 5 peaks representing 5 different types of steroids presence in the leaf extract *Ipomea sagittifolia* were illustrated in Figure 4, 5 & Table 3, 4). The Purple bands were resulted shown the presence of steroids in the crude extract. For saponins at 254nm had shown 1 peak (R_f Value: 0.947; Peak height (H): 0.1323) and 1 peak (R_f Value: 0.944; Peak height (H): 0.2584) respectively had shown in Figure 6 to 9 & Table 5, 6). After derivatization at 366 nm had shown 5 and 7 peaks representing 7 different types of steroids presence in the leaf extract *Ipomea sagittifolia*. All the blue colored bands showed the presence of saponins in the crude extract.Study on coumarins at 254 shown 1 peak (R_f Value: 0.816; Peak height (H): 0.0143) and (R_f Value: 0.819; Peak height (H): 0.0280) and 366 nm had shown 7, 10 peaks representing 10 different types of steroids presence in the leaf extract *Ipomea sagittifolia* given in Figure 10 to 13 & Table 7 to 10. The studies had shown that no blue or green fluorescence after derivatization for the extract under 366nm. These Rf values representing the existence of steroids, saponins and coumarins. The R_f values and peak are determination by HPTLC helps to find out the type of phytoconstituents present in the plant along with their concentration. The separated compounds was well seen had shown in Figure 1 on the HPTLC plates, made visualized against UV of wavelengths 254 nm and 366 nm respectively. In drug development, plant derived phytoconstituents plays an important role [10]. For the treatment of acute and chronic diseases many medicinal plants were used [11]. From prehistoric times, plants have been used for the therapeutic purpose [12].

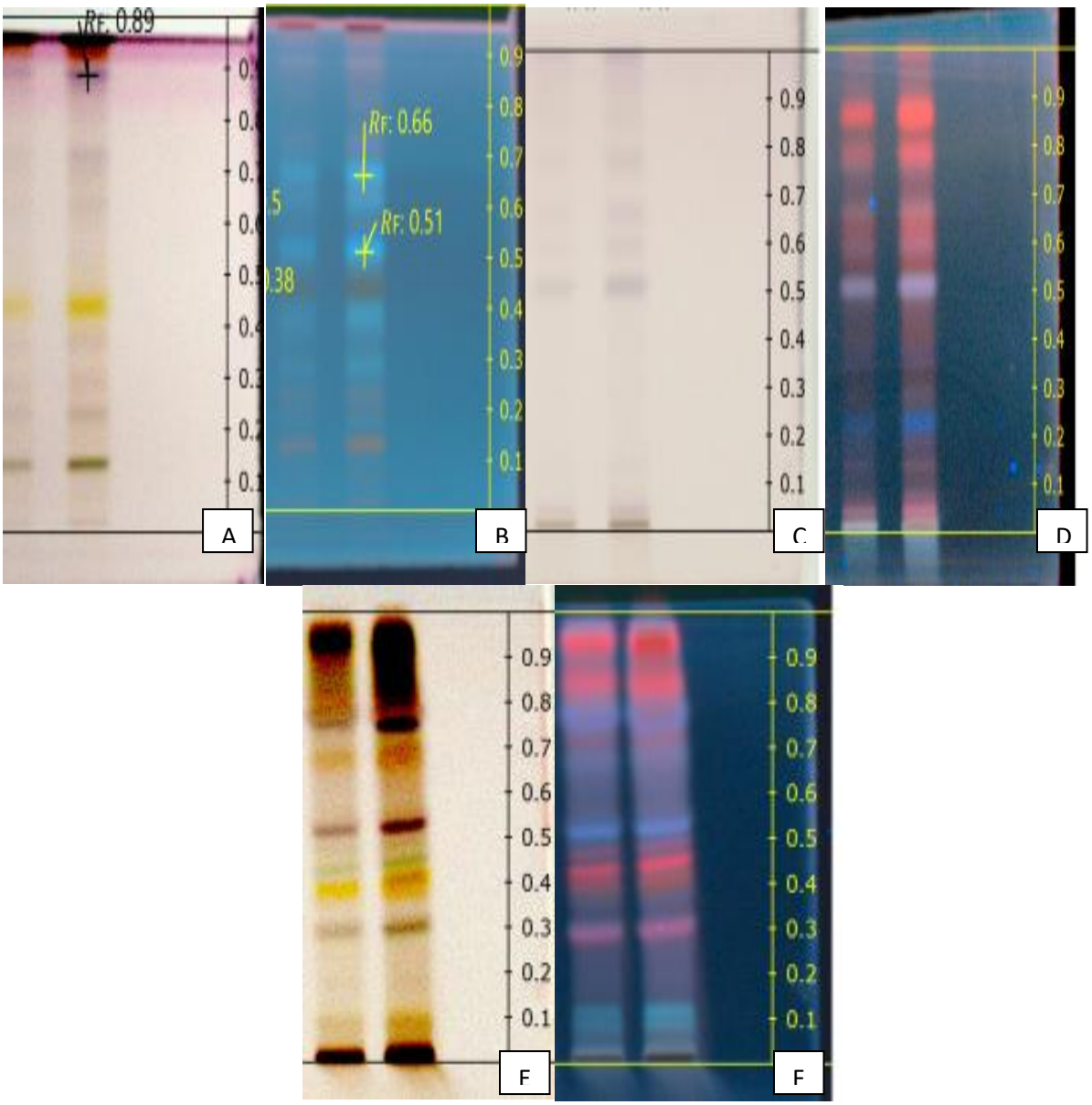

Figure 1:HPTLC plates A, B: Steroids C, D: Saponins and E, F: Coumarins showing developed bands

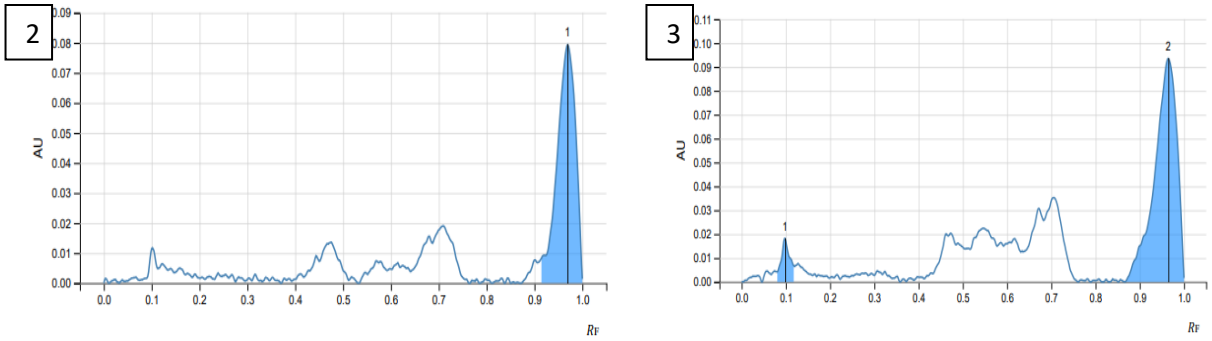

Figure 2 & 3: HPTLC fingerprint analysis for steroids before derivatization scanned at 264 & 366 nm

Table 1. HPTLC fingerprint analysis for steroids showing R_f values and Peak Height (H) at 254 nm:

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.915	0.0083	0.969	0.0794	100.00	1.000	0.0014	0.00354	100.00	No

Table 2. HPTLC fingerprint analysis for steroids showing R_f values and Peak Height (H) at 366 nm:

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.081	0.0042	0.098	0.0182	16.29	0.119	0.0065	0.00042	7.09	No
2	0.866	0.0000	0.965	0.0935	83.71	1.000	0.0017	0.00546	92.91	No

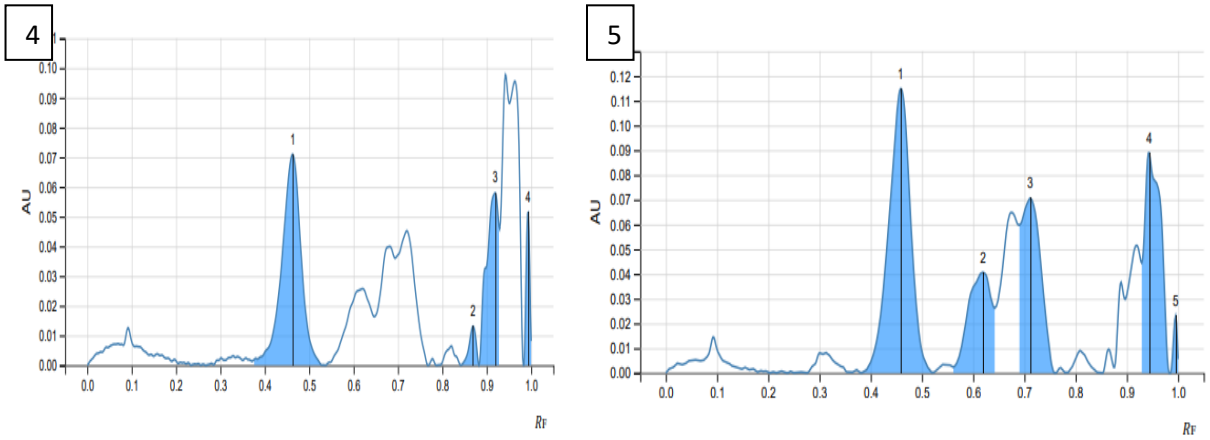

Figure 4 & 5: HPTLC fingerprint analysis for steroids after derivatization scanned at 264 & 366 nm

Table 3. HPTLC fingerprint analysis for steroids before derivatization showing R_f values and Peak Height (H) at 254 nm:

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.376	0.0019	0.463	0.0709	36.68	0.529	0.0000	0.00358	59.00	No
2	0.842	0.0000	0.869	0.0131	6.78	0.882	0.0000	0.00024	3.89	No
3	0.882	0.0000	0.919	0.0580	29.98	0.927	0.0468	0.00176	28.96	No
4	0.984	0.0000	0.994	0.0514	26.56	1.000	0.0081	0.00049	8.15	No

Table 4. HPTLC fingerprint analysis for steroids after derivatization showing R_f values and Peak Height (H) at 366 nm:

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.382	0.0000	0.458	0.1148	33.98	0.521	0.0000	0.00563	39.10	No
2	0.561	0.0023	0.619	0.0406	12.01	0.642	0.0262	0.00214	14.84	No
3	0.689	0.0597	0.711	0.0706	20.90	0.760	0.0000	0.00321	22.28	No
4	0.929	0.0445	0.944	0.0890	26.33	0.982	0.0000	0.00324	22.48	No
5	0.985	0.0000	0.995	0.0229	6.78	1.000	0.0056	0.00019	1.31	No

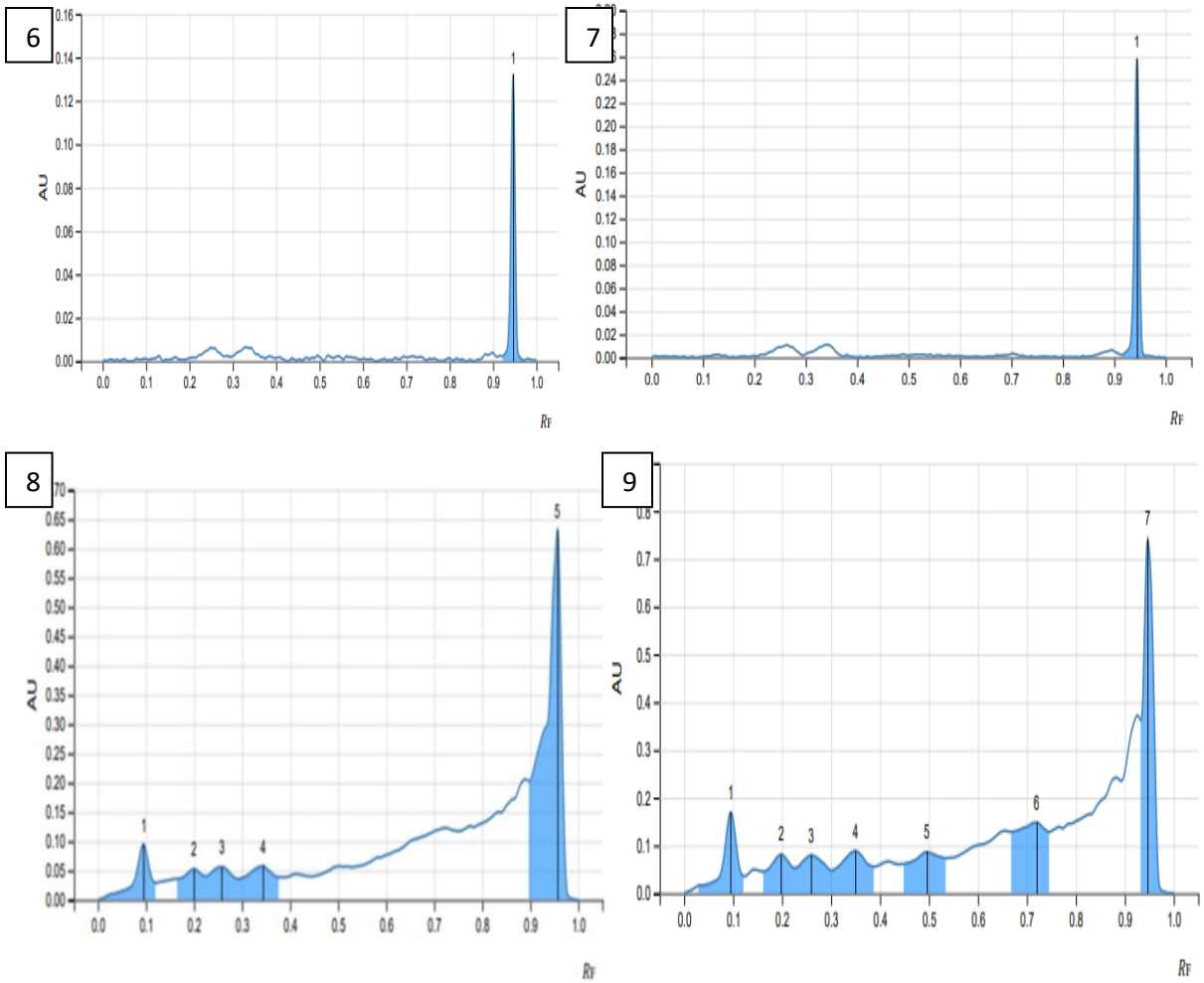

Figure 6, 7, 8 & 9: HPTLC fingerprint analysis for Saponins before and after derivatization scanned at 264 &366 nm

Table 5. HPTLC fingerprint analysis for saponins before derivatization showing R_f values and Peak Height (H) at 254 nm:

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.000	0.0000	0.095	0.0944	10.58	0.121	0.0287	0.00365	9.24	No
2	0.160	0.0357	0.200	0.0526	5.89	0.224	0.0402	0.00281	7.11	No
3	0.224	0.0402	0.258	0.0563	6.31	0.292	0.0356	0.00321	8.13	No
4	0.292	0.0356	0.344	0.0579	6.49	0.379	0.0384	0.00405	10.24	No
5	0.897	0.2028	0.956	0.6308	70.72	0.997	0.0000	0.02580	65.28	No

Table 6. HPTLC fingerprint analysis for saponins after derivatization showing R_f values and Peak Height (H) at 366 nm:

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.027	0.0153	0.095	0.1695	12.15	0.123	0.0356	0.00578	10.22	No
2	0.161	0.0454	0.198	0.0813	5.83	0.226	0.0527	0.00413	7.31	No
3	0.226	0.0527	0.260	0.0799	5.72	0.302	0.0478	0.00498	8.81	No
4	0.302	0.0478	0.350	0.0887	6.36	0.389	0.0556	0.00602	10.66	No
5	0.439	0.0606	0.495	0.0860	6.16	0.537	0.0732	0.00727	12.86	No
6	0.668	0.1286	0.719	0.1481	10.62	0.745	0.1284	0.01066	18.87	No
7	0.932	0.3623	0.947	0.7417	53.16	0.992	0.0004	0.01767	31.27	No

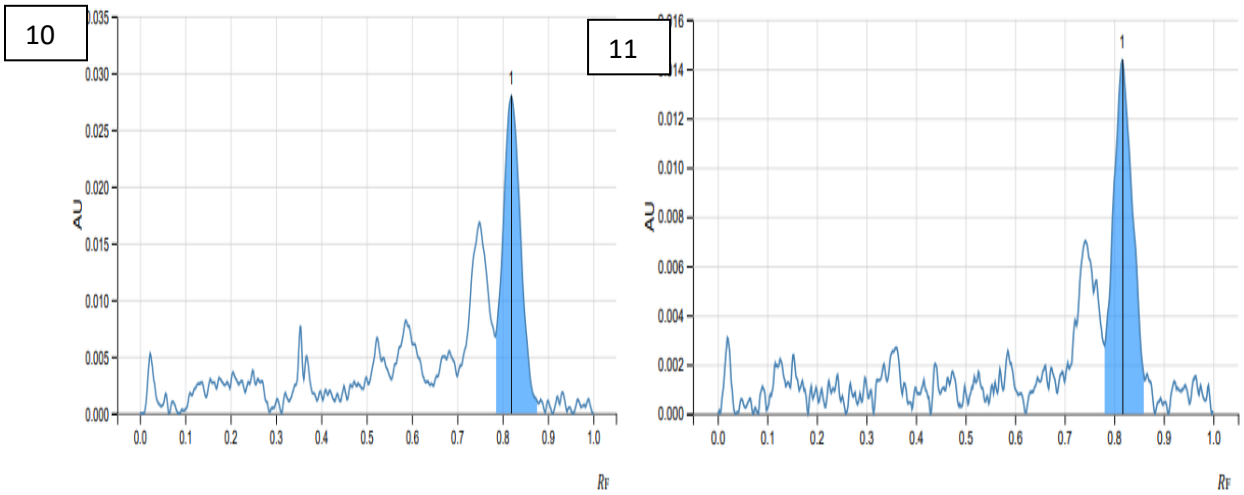

Figure 10 & 11: HPTLC fingerprint analysis for Saponins before derivatization scanned at 264 &366nm

Table 7. HPTLC fingerprint analysis for Coumarins before derivatization showing R_f values and Peak Height (H) at 254 nm:

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.781	0.0028	0.816	0.0143	100.00	0.861	0.0014	0.00065	100.00	No

Table 8. HPTLC fingerprint analysis for Coumarins after derivatization showing R_f values and Peak Height (H) at 366 nm:

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.784	0.0068	0.819	0.0280	100.00	0.879	0.0009	0.00130	100.00	No

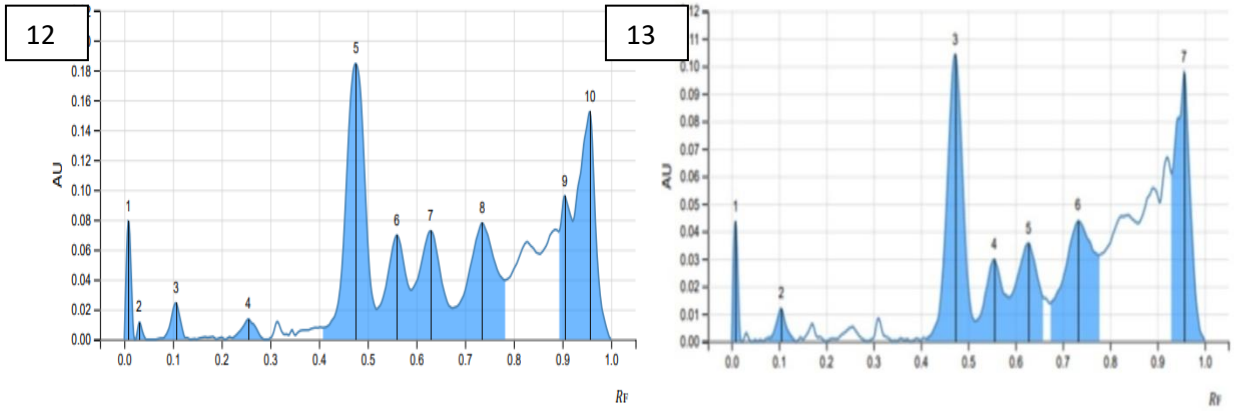

Fig.12 & 13: HPTLC fingerprint analysis for coumarins after derivatization scanned at 254 nm & 366nm

Table 9. HPTLC fingerprint analysis for coumarins before derivatization showing R_f values and Peak Height (H) at 254 nm

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.000	0.0000	0.008	0.0437	11.92	0.019	0.0000	0.00047	3.13	No
2	0.071	0.0006	0.105	0.0119	3.25	0.135	0.0000	0.00029	1.95	No
3	0.411	0.0009	0.473	0.1042	28.41	0.515	0.0073	0.00406	27.13	No
4	0.515	0.0073	0.555	0.0298	8.12	0.585	0.0160	0.00135	9.03	No
5	0.585	0.0160	0.627	0.0356	9.71	0.660	0.0156	0.00189	12.63	No
6	0.673	0.0137	0.732	0.0438	11.94	0.777	0.0313	0.00325	21.75	No
7	0.929	0.0610	0.956	0.0978	26.65	1.000	0.0001	0.00365	24.38	No

Table 10. HPTLC fingerprint analysis for Coumarins after derivatization showing R_f values and Peak Height (H) at 366 nm

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.000	0.0000	0.008	0.0789	10.11	0.021	0.0000	0.00089	2.81	No
2	0.023	0.0000	0.031	0.0114	1.46	0.045	0.0000	0.00012	0.37	No
3	0.069	0.0002	0.106	0.0243	3.11	0.126	0.0010	0.00053	1.68	No
4	0.221	0.0009	0.255	0.0136	1.74	0.287	0.0000	0.00044	1.38	No
5	0.408	0.0078	0.476	0.1843	23.62	0.518	0.0202	0.00847	26.70	No
6	0.518	0.0202	0.560	0.0697	8.93	0.589	0.0330	0.00321	10.11	No
7	0.589	0.0330	0.629	0.0725	9.29	0.674	0.0209	0.00401	12.64	No
8	0.674	0.0209	0.735	0.0777	9.96	0.782	0.0395	0.00532	16.75	No
9	0.892	0.0721	0.905	0.0960	12.30	0.921	0.0792	0.00249	7.84	No
10	0.921	0.0792	0.956	0.1521	19.49	1.000	0.0001	0.00626	19.73	No

In considering the demand of plants, it is needed for a rapid analytical technique for the development of phytomedicines [13]. Hence the objective of the current work is to analyze the class of compounds by HPTLC finger print analysis of the phytoconstituents like steroids, coumarins and saponins in *Ipomea sagittifolia*. A steroid conciliates a number of responses in biological system. For instance, cholesterol, is an essential precursor for the production of other steroids viz., corticoids, androgens, estrogens etc., The steroids are widely used in the treatment of many ailments like allergic reactions, arthritis etc., some malignancies and diseases resulting from hormone deficiencies or abnormal production [14]. Coumarins, occurs naturally and also used as a precursor molecule in synthesis of anticoagulants [15]. Earlier studies were reported that coumarin is an antioxidant and able to chelate metal ions [16]. It also exerts action on immune system, lymphedema, antiseptic, skin etc., [17-20]. The HPTLC analysis of methanolic leaf extract of *Ipomea sagittifolia* revealed the presence of steroids, saponins and coumarins by observing the developed peaks in each chromatogram shown in Figures. 2 to 13. The chromatogramswere acquired at the wavelength 254nm and 366 nm. The resulted Rf values, height and peak area along with percent area of the phytochemicals are illustrated in the tables of chromatogram and each chromatogram were explained in results and discussion part shown in Table 1 to 10. Natural product research as they have high performance in improving nutrition for tissues, antiaging etc Saponins are glycosidic triterpenoids wide spread of occurrence in plant kingdom [21-22]. Saponins results in unique biological activity and exerts therapeutic activities like anti-inflammatory, immunomodulatory etc., [23]. Numerous plants produce secondary metabolites viz., alkaloids, flavanoids, saponins, terpenes, etc., not only used as therapeutic agents but also in cosmetics and pesticide industries. The results of the present study revealed 23, 7, 10 different types of steroids, saponins and coumarins in the leaf of *Ipomea sagittifolia*.

Thus, the current study confirms the traditional medical practices in treatment of many ailments. The results of the current study would serve as evidence for the presence of several known and unknown bioactive compounds viz., steroids, saponins and coumarins with bioactivity explores folkloric usage of this plant. New drugs can be formulated to treat numerous diseases by isolating and identifying these unknown bioactive compounds. Authentication of medicinal plants in aspects of both genetics and chemically is an important criterion for the usage of these botanical in research purposes. In molecular era, taxonomy and morphological characters of the plant helps in plant systematic study and its classification. Apart from these markers, at biochemical, anatomical, cytological and molecular level is used for the classification. HPTLC profile (Chemical profile) of the ethanolic extracts of *Ipomea sagittifolia* supplements and confirms and strengthens the identification of steroids, saponins and coumarins using HPLTC profile in this study. Information of chemical constituents present in the plant helps in chemo-taxonomical classification of the plant.The results of present study revealed the presence of 23, 7, 10 different types of steroids, saponins and coumarins with 23, 7, 10 different R_f values in leaves of *Ipomea sagittifolia*. HPTLC profile of steroids, saponins and coumarin has been revealed the diversity in existence of biochemical level. Hence linear, exact and accurate HPTLC fingerprinting approach can be utilized to further authenticate and characterize the medicinally significant plant. The manufacturer would benefit from the generated HPTLC fingerprints for quality assurance and standardization of herbal mixtures. These biochemical markers for this medicinally significant plant can be used in the pharmaceutical sector and in plant systematic investigations to distinguish the species from the adulterant [24-30]. The findings of the present study are limited to the HPTLC

analysis of *Ipomea sagittifolia* methanolic leaf extract to estimate the presence of different phytochemicals like steroids, saponins and flavonoids from the chromatogram peaks and obtain the peak tables; however, the quantification of these phytochemicals is necessary to study.

The current study revealed the presence of steroids, flavonoids and saponins in *Ipomea sagittifolia* which was used in traditional medicine for various ailments. In drug development, isolated chemical entities and their profiling is very much needed. The HPTLC analysis for methanolic leaf extract of *Ipomea sagittifolia* helpful in chemical profiling and identification of bioactive constituents when their R_f values of these compounds are compared with standards as reference.

Author Contribution

Conceptualization: RP, ALR and ASAMS; Investigation and writing original draft: MSSSS and KBS; review and editing: ALR and ASAMS. All authors have read and agreed to the published version of the manuscript.

Abbreviations

HPTLC: High Performance Thin Layer Chromatography; R_f: Retardation Factor; H: Height; ADC: Automatic Developing Chamber; TLC: Thin Layer Chromatography; ATS: Automatic TLC Sampler

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