Investigation on ethanolic stem extract of *Ipomea sagittifolia* to explore the presence of Saponins by High Performance Thin Layer Chromatography

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Abstract: Background: Saponins are a diverse group of naturally occurring plant compounds that play important roles in various aspects of plant physiology and ecology. An extract composed of several phytoconstituents may be easily analysed and interpreted using high performance thin layer chromatography (HPTLC) fingerprint analysis from a qualitative standpoint. Ipomea sagittifolia (Burn. f.) has a long history of use in traditional medical systems.

Method: It was decided to interpret the phytoconstituents, namely the steroids in Ipomea sagittifolia (Burn. f.), in line with Current Good Manufacturing Practises, which highlight the significance of quality in relation to phytoconstituents, because HPTLC is a very trustworthy method of analysis. In this work, an HPTLC finger print profile for the saponins was made using an ethanolic extract of the stem of Ipomea sagittifolia (Burn. f.). This system includes the Scanner 4, TLC Visualizer, and Linomat 5 Applicator.

Results: When phytoconstituents were evaluated using HPTLC densitometric screening at wavelengths of 366 nm and 540 nm, several peaks could be seen in the chromatogram. By analysing the peak regions, peak heights, and Rf values that were given in the proper tables, the phytochemicals are appraised.

Conclusion: The study revealed the presence of saponins. This information is highly useful in studying chemical profiling and discovering bioactive components when the Rf values of these chemicals are compared with standards as a reference.

Keywords: Saponins, Phytochemical, Morning glory, Metabolites, HPTLC, Analysis.

INTRODUCTION

Saponins are a fascinating group of naturally occurring plant compounds that have a long history of human interaction and use. The term "saponin" is derived from the Latin word "sapo," which means soap, owing to their ability to produce a soapy foam when agitated in water. These compounds are found in a wide variety of plants, including herbs, legumes, grains, and some fruits. [1]The property has been exploited by various cultures for centuries in cleaning, washing, and even in producing frothy beverages.However, beyond their frothy nature, saponins serve essential functions in plants. [2] One of their primary roles is to act as a defense mechanism against herbivores, pathogens, and pests. When predators attempt to feed on a plant rich in saponins, they experience a bitter taste and gastrointestinal distress, leading to reduced feeding and potential deterrence. This defensive function helps the plant to survive and reproduce in the face of various threats. Saponin-rich plant species have

been used in traditional medicine across various cultures for centuries. [3] Traditional uses include treating respiratory ailments, skin conditions, and gastrointestinal disorders. [4] Modern research continues to explore the medicinal potential of saponins, particularly in the areas of anticancer, anti-inflammatory, and immunomodulatory therapies. [5]

A species of flowering plant belonging to the morning glory family (Convolvulaceae), Ipomoea sagittifolia. It is aherbaceous climbing vine that may reach a maximum length of 5 metres and has purple or pink blooms with a white or yellow center. [6] It grows in a variety of environments, including woods, grasslands, wetlands, and disturbed places. It is indigenous to many tropical and subtropical regions of Africa, Asia, and Australia. Some regions of the world also cultivate it as an attractive plant. Ipomoea sagittifolia is also known as Indian potato, arrow-leaf morning glory, and purple heart glory. It is often mistaken for other Ipomoea species, including those with similar shape and range, such Ipomoea sepiaria, Ipomoea marginata, and Ipomoea maxima. Traditional medical practises including Ayurveda, Siddha, Unani, and Chinese medicine all employ *Ipomoea sagittifolia* as a medicinal plant. [7] The plant is said to have a variety of pharmacological properties, including anti-inflammatory, antidiabetic, etc. [8]

By comparing retention factors (Rf values) and spectral traits with those of reference standards or databases, HPTLC may be used to detect the secondary metabolites in plant extracts. [9-11]. The secondary metabolites in plant extracts may also be quantified using HPTLC by quantifying their peak areas or heights and using calibration curves or standard addition techniques. Additionally, plant extracts may be employed with HPTLC to create distinctive fingerprints that represent their chemical richness and variability. Therefore, the goal of the current study is to determine if saponins are present in *Ipomea sagittifolia* stem ethanolic extract using high performance thin layer chromatography.

METHODS

Extraction

The stems of the plant *Ipomea sagittifolia* from Gudlavalleru's surrounds were clipped at the base. The stems were washed and given a week to dry in the sun. The stems were mechanically ground after drying. A rotary evaporator was used to concentrate 100g of stem powder after it had been extracted with ethanol using a Soxhlet equipment.

Instrumentation

HPTLC: HPLTC analysis of plant extracts provides valuable information about the presence and relative abundance of various compounds, which can help in understanding the plant's phytochemical profile and potential pharmacological activities. The technique is widely used in botanical research, pharmaceutical development, and natural product chemistry.

Preparation of Sample: *Ipomea sagittifolia*stemswas extracted with ethanol to obtain 1 mg, further made concentrated in 1 ml of methanol and filtered.

Development Chamber: After applying the sample, the HPTLC plate was kept in a development chamber containing mobile phase. Chloroform, Glacial acetic acid, Methanol, Water (64:32:12:8 v/v/v/v) were utilised in the mobile phase for saponins testing. 20 minutes pass before saturation.

Derivatization: The created plate for steroids phenols is derivatized 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. Add 1ml of anisaldehyde after allowing the liquid to cool to room temperature [12-13].

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

RESULTS

The HPTLC analysis of theethanolic stem extract of *Ipomea sagittifolia* revealed the presence of saponinsby examining the produced peaks in each chromatogramwhen scanned at 254nm, 366nm and 540nmof 2.0µl and 5.0µl each shown the presence of saponins shown in Figure 1 & 2.Hence further the plates were treated with derivatized reagent inorder to ensure the clarification. The chromatograms were acquired at wavelengths of 366nm

and 540 nm of sample volume at 2.0µl and 5.0µl (Track 1 & 2). The derivatized plates (Plate a, b and c) containing the samples were analysed of both volumes 2.0µl and 5.0µl. The tables of chromatograms show the phytochemicals peak areas, heights, 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 saponins. The chromatograms resulted for finger printing analysis of saponinshad shown well differentiated one peak at R_f 0.952 at peak height 0.3578 (Sample Volume: 2.0µl/Track 1) and 1 peak at shown R_f0.945 at peak height 0.4974 (Sample Volume: 5.0µl/Track 2) at 254nm shown in Figure 3&4. In derivatized plate b, the analysis shown that 5 peaks at R_f0.187, 0.515, 0.682, 0.895, 0.966at a peak height of 0.0970, 0.0492, 0.682, 0.895, 0.966 (Sample Volume: 2.0µl/Track 1) & shown 7 peaks at R_f0.187, 0.410, 0.513, 0.681, 0.719, 0.885, 0.958 at peak height 0.1717, 0.0784, 0.0865, 0.2334, 0.1966, 0.3792, 0.7724sample volume 5.0 µl (Track 2) at 360nm shown in Figure 5&6. In derivatized plate b, the analysis shown that 8 peaks at R_f0.094, 0.200, 0.258, 0.342, 0.413, 0.724, 0.885, 0.953at a peak height of 0.0760, 0.0369, 0.0368, 0.0340, 0.0379, 0.0763, 0.1901, 0.6268 (Sample Volume: 2.0µl/Track 1) & shown 9 peaks at R_f0.095,0.197,0.260,0.347,0.415,0.618,0.719,0.877,0.945 at peak height 0.1384, 0.0588, 0.0570, 0.0621, 0.0704, 0.0810, 0.1151, 0.2664, 0.7324 sample volume 5.0 µl (Track 2) at 540nm shown in Figure 6&7.

DISCUSSION

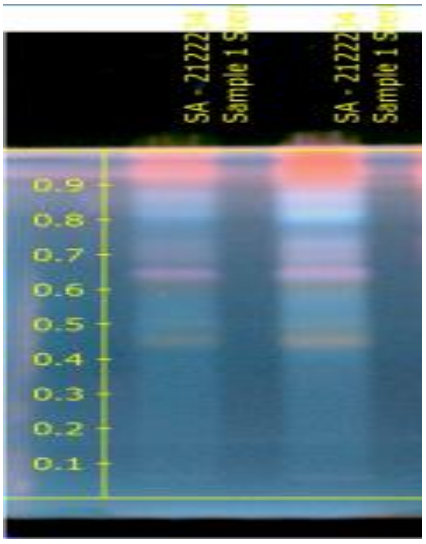

Fig.1 Derivatized Plates showing well differentiated Bands at White & Remission

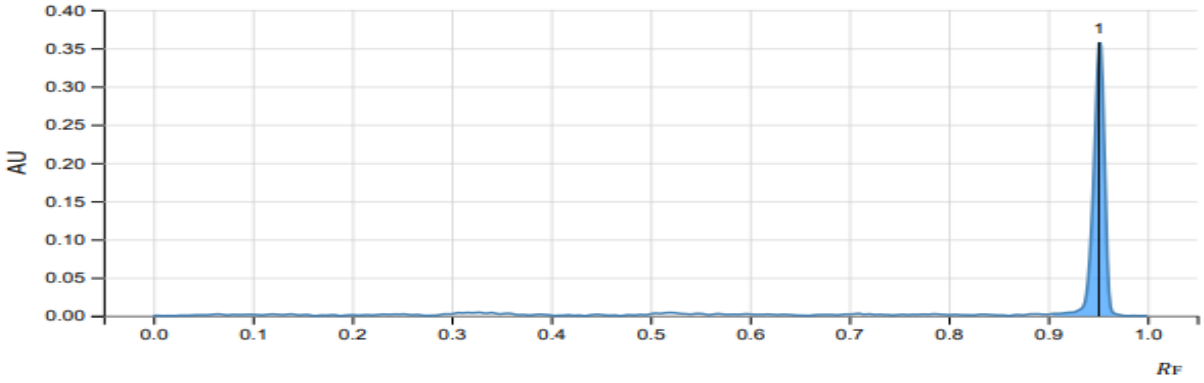

Figure2: HPTLC showing peak at 254nm at sample Volume 2.0µl after derivatization denotes presence of saponins

Table 1: HPTLC at 254nm at sample Volume 2.0µl representing R_f and Peak height for saponins:

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.902	0.0022	0.952	0.3578	100.00	0.981	0.0000	0.00465	100.00	No

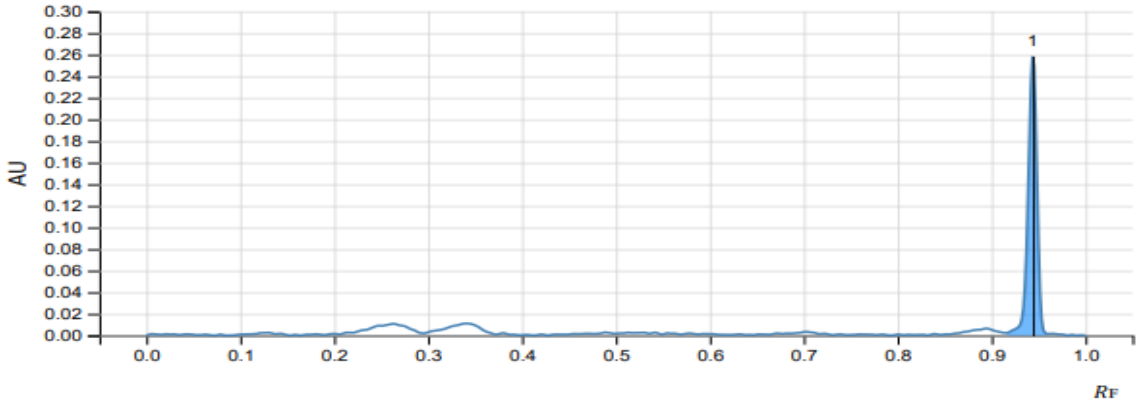

Figure 3: HPTLC showing peak at 254nm at sample Volume 5.0µl after derivatization denotes presence of saponins

Table 2: HPTLC at 254nm at sample Volume 5.0µl representing R_f and Peak height for saponins:

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.915	0.0023	0.944	0.2584	100.00	0.961	0.0015	0.00286	100.00	No

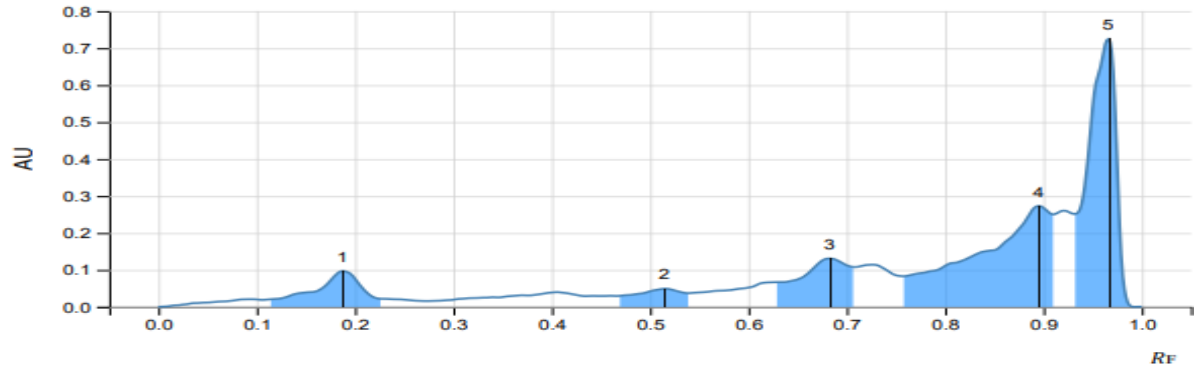

Figure4: HPTLC showing peak at 360nm at sample Volume 2.0µl after derivatization denotes presence of saponins

Table 3: HPTLC at 360nm at sample Volume 2.0µl representing R_f and Peak height for saponins

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.106	0.0188	0.187	0.0970	7.61	0.231	0.0217	0.00578	9.01	No
2	0.466	0.0295	0.515	0.0492	3.86	0.539	0.0369	0.00287	4.48	No
3	0.627	0.0667	0.682	0.1308	10.26	0.708	0.1077	0.00799	12.46	No
4	0.756	0.0829	0.895	0.2730	21.43	0.910	0.2500	0.02400	37.44	No
5	0.932	0.2513	0.966	0.7243	56.84	0.992	0.0000	0.02347	36.61	No

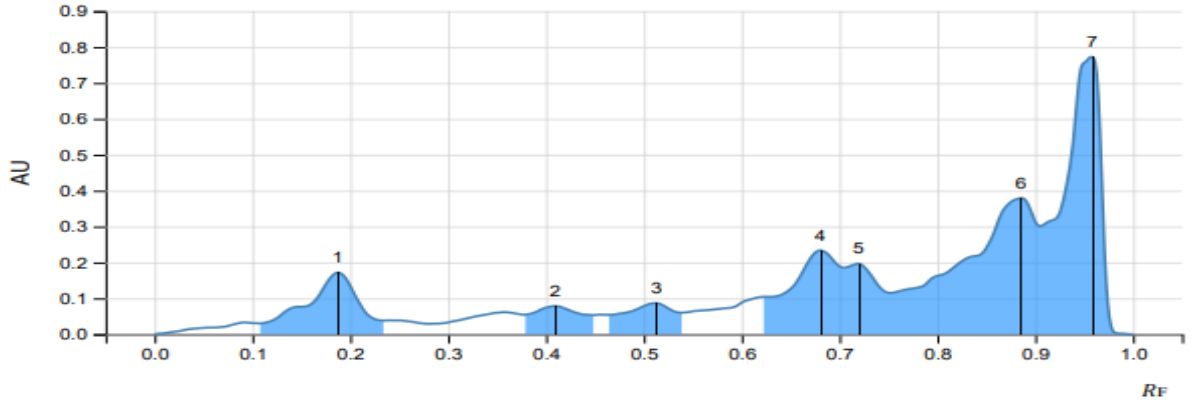

Figure 5: HPTLC showing peak at 360nm at sample Volume 5.0µl after derivatization denotes presence of saponins

Table 4: HPTLC at 360nm at sample Volume 5.0µl representing R_f and Peak height for saponins

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.106	0.0302	0.187	0.1717	8.95	0.234	0.0381	0.01097	9.86	No
2	0.377	0.0544	0.410	0.0784	4.09	0.448	0.0531	0.00461	4.14	No
3	0.465	0.0540	0.513	0.0865	4.51	0.539	0.0599	0.00515	4.62	No
4	0.621	0.1040	0.681	0.2334	12.17	0.705	0.1851	0.01390	12.49	No
5	0.705	0.1851	0.719	0.1966	10.25	0.752	0.1150	0.00771	6.93	No
6	0.752	0.1150	0.885	0.3792	19.77	0.905	0.3007	0.03466	31.14	No
7	0.905	0.3007	0.958	0.7724	40.27	0.997	0.0000	0.03430	30.82	No

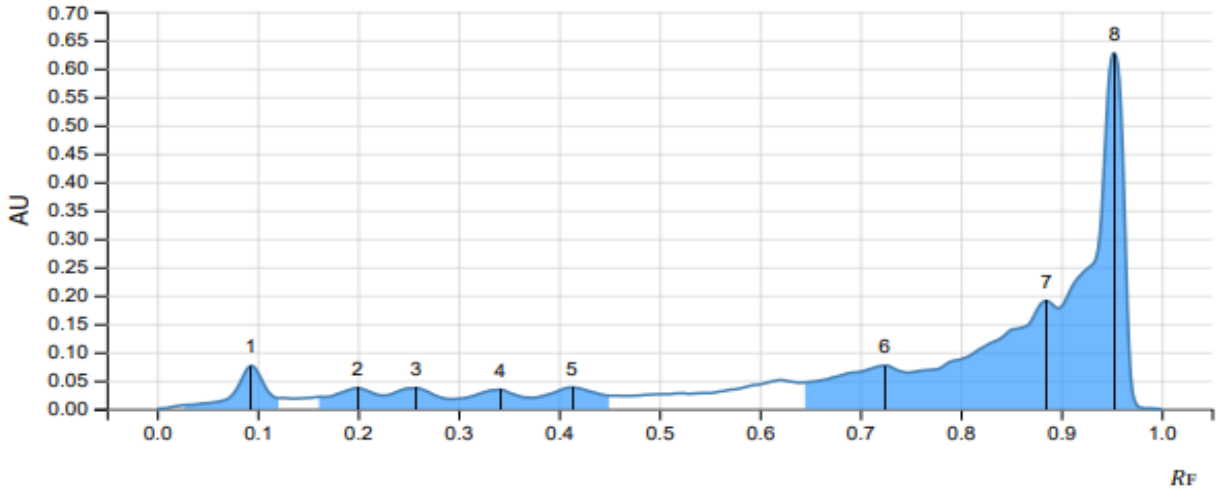

Figure 6: HPTLC showing peak at 540nm at sample Volume 2.0µl after derivatization denotes presence of saponins

Table 5: HPTLC at 540nm at sample Volume 2.0µl representing R_f and Peak height

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.024	0.0061	0.094	0.0760	6.82	0.121	0.0190	0.00273	4.68	No
2	0.158	0.0207	0.200	0.0369	3.31	0.224	0.0238	0.00186	3.19	No
3	0.224	0.0238	0.258	0.0368	3.30	0.294	0.0171	0.00196	3.36	No
4	0.294	0.0171	0.342	0.0340	3.05	0.374	0.0196	0.00203	3.48	No
5	0.374	0.0196	0.413	0.0379	3.40	0.456	0.0231	0.00239	4.09	No
6	0.639	0.0461	0.724	0.0763	6.85	0.747	0.0639	0.00666	11.41	No
7	0.747	0.0639	0.885	0.1901	17.05	0.897	0.1773	0.01711	29.31	No
8	0.897	0.1773	0.953	0.6268	56.23	0.984	0.0010	0.02362	40.47	No

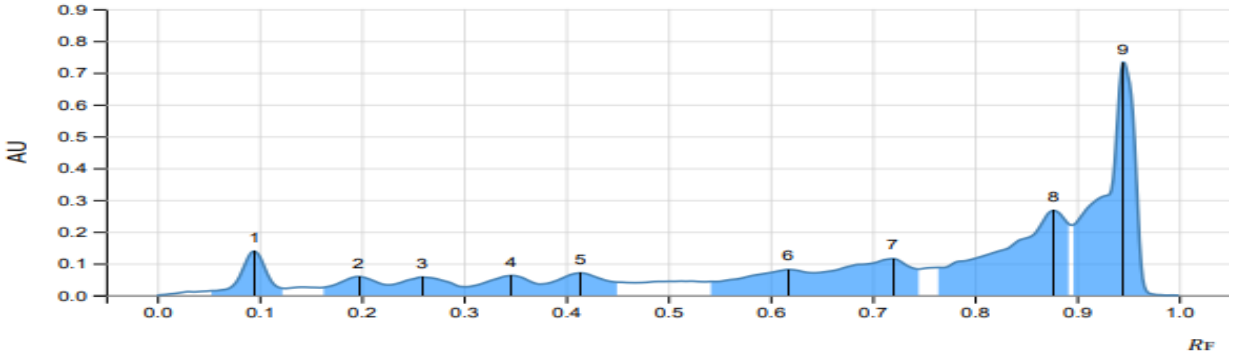

Figure 7: HPTLC showing peak at 540nm at sample Volume 2.0µl after derivatization denotes presence of saponins

Table 6: HPTLC at 540nm at sample Volume 5.0µl representing R_f and Peak height

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.048	0.0130	0.095	0.1384	8.75	0.124	0.0216	0.00424	5.21	No
2	0.160	0.0238	0.197	0.0588	3.72	0.227	0.0327	0.00287	3.53	No
3	0.227	0.0327	0.260	0.0570	3.60	0.302	0.0266	0.00329	4.03	No
4	0.302	0.0266	0.347	0.0621	3.93	0.374	0.0352	0.00335	4.11	No
5	0.374	0.0352	0.415	0.0704	4.45	0.453	0.0410	0.00417	5.11	No
6	0.539	0.0428	0.618	0.0810	5.12	0.642	0.0706	0.00652	8.01	No
7	0.642	0.0706	0.719	0.1151	7.28	0.745	0.0825	0.00959	11.77	No
8	0.761	0.0870	0.877	0.2664	16.84	0.894	0.2214	0.02105	25.83	No
9	0.895	0.2204	0.945	0.7324	46.31	0.987	0.0000	0.02640	32.40	No

These Rf values demonstrate the presence of saponins. Utilising 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 254 nm, 366 nm, and 540 nm, respectively. Since they have been utilised for treating both acute and chronic ailments since the beginning of time, plant-derived phytoconstituents are essential for the production of medicines. [14] Due to the demand for plants, a quick analytical approach is necessary for the study and development of phytomedicines. [15]

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 use of HPTLC to the investigation of drug formulations, bulk medicines, and natural materials will increase in the future. [16] The primary goal of this research is to use HPTLC finger print analysis to identify botanical components including glycosides, essential oils, and tannins.

Saponins from plants have gained significant attention in pharmacology due to their diverse and promising pharmacological activities. These natural compounds offer potential therapeutic benefits and have been studied extensively for their various biological properties like Anticancer. Saponins have demonstrated potential as anticancer agents by inducing apoptosis (programmed cell death) and inhibiting tumor cell growth. They have shown activity against various cancer types, making them promising candidates for cancer treatment research. [17]

Certain saponins exhibit cardioprotective properties by reducing cholesterol levels, improving lipid profiles, and regulating blood pressure. They have been investigated for their potential in managing cardiovascular diseases. [18]Saponins can modulate the immune system, enhancing immune responses and providing potential benefits in immune-related disorders and diseases. [19]Saponins have shown antiviral and antimicrobial activities, inhibiting the growth and replication of various viruses, bacteria, and fungi. These properties can be valuable in combating infectious diseases. [20]

Some saponins exhibit neuroprotective properties, potentially protecting neurons from damage and providing benefits in neurodegenerative diseases. [21]Saponins have demonstrated anti-inflammatory and analgesic activities, making them potential candidates for managing inflammatory conditions and pain. [22]Saponins from plants represent a valuable source of bioactive compounds with promising pharmacological potential. Ongoing research continues to uncover their mechanisms of action and explore their applications in various medical fields.

The findings of this study showed that *Ipomea sagittifolia* leaves contain a variety of saponin kinds. The current study thus supports the fact that traditional medicine is successful in treating a wide range of illnesses.

Many plants contain secondary metabolites that are used as therapeutic agents as well as in the cosmetics and pesticide industries. These substances include steroids, alkaloids, flavanoids, saponins, terpenes, and many more. The authenticity of medicinal plants in terms of both genetic and chemical characteristics is a critical need for the use of these botanicals in research. In the era of molecular biology, taxonomy and the morphological traits of plants are helpful in the systematic study of plants and their classification. The classification is also based on biochemical,

anatomical, cytological, and molecular characteristics. The HPTLC profile (Chemical profile) of ethanolic extracts of *Ipomea sagittifolia* complements, improves, and confirms the study's HPLTC profile's detection of steroids. The understanding of the chemical components that are present aids in the chemo-taxonomical classification of the plant.

The current study's findings indicated the existence of saponins. The HPTLC profile of steroids has shown the range of biochemical levels that can occur. A linear, precise, and accurate HPTLC fingerprinting strategy may then be usd to further characterise 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 steroids from chromatogram peaks and obtain peak tables. However, the research still necessitates quantifying these phytoconstituents.

CONCLUSION

According to the present study, the *Ipomea sagittifolia* plant apparently contains steroids that are employed in traditional medicine to treat a number of disorders. 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.

List of Abbreviations

HPTLC: High Performance Thin Layer Chromatography; ml: milli liters;TLC: Thin Layer Chromatography.

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DOI: https://doi.org/10.15379/ijmst.v10i4.2210