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Assessment of Steroids in ethanolic stem extract of Morning glory by High Performance Thin Layer Chromatography

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Abstract

HPTLC fingerprint analysis is simple to analyse and interpret an extract made up of various phytoconstituents from a qualitative perspective. Ipomea sagittifolia (Burn. f.) has been employed in traditional medicine systems for a very long time. Given that HPTLC is a very reliable method of analysis, it was chosen to interpret the phytoconstituents, namely the steroids in Ipomea sagittifolia (Burn. f.), in accordance with Current Good Manufacturing Practises, which emphasise the importance of quality in regard to phytoconstituents. In this study, Ipomea sagittifolia (Burn. f.) stem ethanolic extract was used to create an HPTLC fingerprint profile for the steroids. This system included the Linomat 5 Applicator, TLC Visualizer, and Scanner 4. Numerous peaks were visible in the chromatogram when phytoconstituents were assessed using HPTLC densitometric screening at wavelengths of 366 nm and 540 nm. The phytochemicals are evaluated by interpreting the peak areas, peak heights, and Rf values that were reported in the appropriate tables. The research made steroid use evident. When the Rf values of these substances are contrasted with standards as a reference, this information is very helpful in exploring chemical profiling and identifying bioactive ingredients.

Keywords: Steroids, Metabolites, HPTLC-fingerprints, Chromatogram

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

1. Introduction

Steroids are organic compounds are widely distributed in nature and have various biological functions in animals and plants. Plant steroids are a diverse group of natural products that are derived from squalene, a hydrocarbon that is produced from acetic acid via the acetate-mevalonate pathway. Plant steroids are sterols and brassinosteroids. Besides plant sterols and brassinosteroids, there are other types of plant steroids that have been isolated from various sources and have shown interesting biological activities viz., Diosgenin from fenugreek (*Trigonella foenum-graecum*), wild yam (*Dioscorea villosa*), and sarsaparilla (Smilax spp.). Solasodine from eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*), and potato (*Solanum tuberosum*). Boswellic acid from resin of Boswellia serrata (Indian frankincense. Glycyrrhizin from *Glycyrrhiza glabra* (licorice), Guggulsterones from the resin of *Commiphora mukul* (guggul), Withanolides from Withania somnifera (ashwagandha). plant steroids are a diverse and fascinating group of natural compounds that have various biological functions and pharmacological activities. They have potential applications in medicine, agriculture, and biotechnology [1].

Ipomoea sagittifolia is a species of flowering plant in the morning glory family (Convolvulaceae). It is a climbing herbaceous vine that grows up to 5 meters long and produces purple or pink flowers with a white or yellow center [2]. It is native to many tropical and subtropical regions of Africa, Asia, and Australia, where it grows in various habitats, such as forests, grasslands, wetlands, and disturbed areas. It is also cultivated as an ornamental plant in some parts of the world. *Ipomoea sagittifolia* has several common names, such as purple heart glory, arrow-leaf morning glory, and Indian potato. It is sometimes confused with other species of Ipomoea, such as Ipomoea sepiaria, *Ipomoea marginata*, and *Ipomoea maxima*, which have similar morphology and distribution. However, Ipomoea sagittifolia can be distinguished by its arrow-shaped leaves with purple blotches in the center and its larger flowers with a distinct corolla tube. *Ipomoea sagittifolia* is used as a medicinal plant in traditional systems of medicine, such as Ayurveda, Siddha, Unani, and Chinese medicine [3]. The plant is reported to have various

pharmacological properties, such as anti-inflammatory, antidiabetic, antihypertensive, antitumor, antiviral, antibacterial, antifungal, antiprotozoal, antihelminthic, cytotoxic, immunomodulatory, hepatoprotective, neuroprotective, adaptogenic, anti-stress, anti-aging, hormone-regulating, contraceptive, abortifacient, aphrodisiac, diuretic, laxative, purgative, and emetic effects [4]. HPTLC can be used to identify the secondary metabolites in plant extracts by comparing their retention factors (Rf values) and spectral characteristics with those of reference standards or databases [5,6,7]. HPTLC can also be used to quantify the secondary metabolites in plant extracts by measuring their peak areas or heights and applying calibration curves or standard addition methods. HPTLC can also be used to generate characteristic fingerprints of plant extracts that reflect their chemical diversity and variability. Hence the current work is to identifiy the presence of steroids in stem ethanolic extract of *Ipomea sagittifolia* by using High Performance Thin Layer Chromatography.

2. Experimental Method

2.1. Extraction

The stems were made cut at the base of the plant *Ipomea sagittifolia* from the surroundings of Gudlavalleru. Stems were cleaned and allowed to dry in the sun for a week. The dried stems were ground mechanically. 100g of stem powder were extracted with ethanol using a Soxhlet apparatus before being concentrated with a rotary evaporator.

2.2. Instrumentation

The CAMAG HPTLC system included ATS 4 applicator, TLC visualizer and scanner 4.

2.3. HPTLC Specifications

2.3.1. Preparation of Sample

Ipomea sagittifolia stems were extracted with ethanol to yield 1 mg, which was then concentrated in 1 ml of methanol and filtered.

2.3.2. Development Chamber

The HPTLC plate was held in a development chamber with mobile phase after the sample application. For steroids mobile phase Toluene: Ethyacetate: Methanol (4:4:1 v/v/v) were used. Saturation time is 20 min.

2.3.3. Derivatization

For steroids phenols the developed plate is derivatized by placing 170 ml of methanol in 200ml glass bottle and cooled it down in water-ice cube bath. Slowly 20ml of acetic acid and 10 ml of sulphuric acid and mix well. Allow the mixture to cool at room temperature, then add 1ml of anisaldehyde [8,9].

2.3.4. Visualization

The produced bands were efficiently scanned at wavelengths of 366 nm and 540nm with the help of TLC Scanner 4.

3. Results and Discussion

The HPTLC analysis of the ethanolic stem extract of *Ipomea sagittifolia* revealed the presence of steroids by examining the produced peaks in each chromatogram when scanned at 366nm and 540nm of 2.0µl and 5.0 µl each shown the presence of steroids (Figs. 1 to 4). 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 5.0 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 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 steroids. The generated band on the HPTLC plate examined around 366nm and 540nm are shown in Figure 1. The derivatized plates were shown in Fig. no.3. The chromatograms resulted for finger printing analysis of steroids had shown well differentiated one peak at R_f 0.976 at peak height 0.0415 (Sample Volume: 2.0µl/Track 1) and 2 peaks at shown R_f - 0.865, 0.973 at peak height 0.0215, 0.0886 (Sample Volume: 5.0µl/Track 2) fig 1, 2 at 540nm. In derivatized plate b, the analysis shown that 3 peaks at R_f 0.413, 0.626, 0.940 at peak height 0.0366, 0.0394, 0.0629 (Sample Volume: 2.0µl/Track 1) & shown 6 peaks at R_f 0.411, 0.629, 0.866, 0.918, 0.968, 0.990 at peak height 0.0754, 0.0859, 0.0179, 0.0617, 0.0882, 0.0198 sample volume 5.0 µl (Track 2) at 366nm. These Rf values show that steroids are present. It is simpler to identify the kind and quantity of botanical elements present in the plant using the Rf and peak values computed by HPTLC. On the HPTLC plates, which were rendered visible under UV of wavelengths 366 nm and 540 nm, respectively, the separated compounds were clearly apparent. (Fig. 1). Plant-derived phytoconstituents are crucial for the manufacturing of pharmaceuticals since they have been used for treating both acute and chronic disorders since the beginning of time [9–10]. For the study and development of phytomedicines, a rapid analytical method is required due to the need for plants [10–11]. The study of phytochemicals and biological molecules, the measurement of pharmaceuticals and active ingredients, formulation fingerprinting, and the detection of adulterants in formulations are all examples of applications for HPTLC. Chemicals of forensic importance can be located using HPTLC. The use of a hyphen in HPTLC-MS, HPTLC-FTIR, and HPTLC-Scanning Diode Laser, in addition to other cutting-edge HPTLC-related techniques, has made HPTLC a powerful analytical tool. The analysis of drug formulations, bulk pharmaceuticals, and natural materials will benefit more from the application of HPTLC in the future, according to experts [12]. Thus, the main objective of this research is to identify the botanical elements, such as glycosides, essential oils, and tannins, using HPTLC finger print analysis. Steroids include drugs used to relieve swelling and inflammation, such as prednisone and cortisone; vitamin D; and some sex hormones, such as testosterone and estradiol [13, 14] For this review, Steroids were detected in most plant species like *citrus fruit juice*, peel and *juice of citrus Medica*, *Flaxseeds*, *Nigella sativa*, *Ocimum gratissimum Linn* leaf, *Syzygium guineans* root, and Root and Stem bark of *Vernonia amygdalina* in common plant species while in some plant species were shown variable result that depends on the given solvents and not totally detected in the part of the plant like *Rhamnus prinoides* root [15].

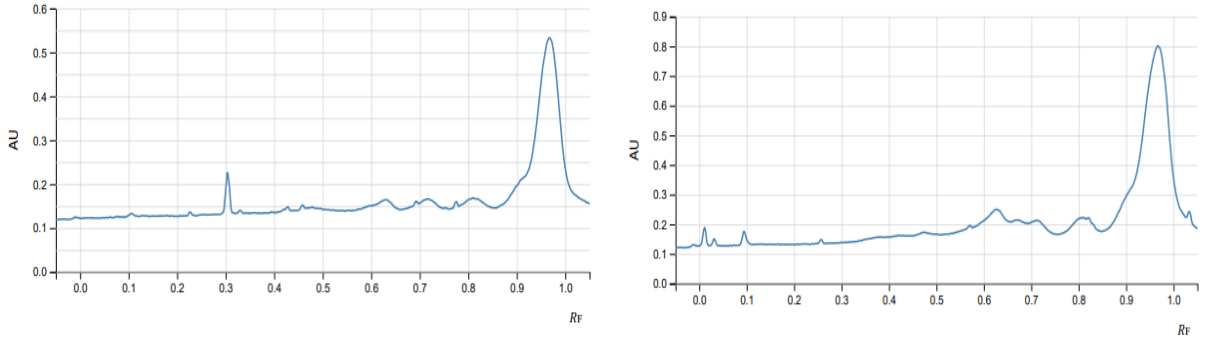


Figure 1. Peaks shown when scanned at 366nm 2.0µl (Track 1) & 5.0µl (Track 2)

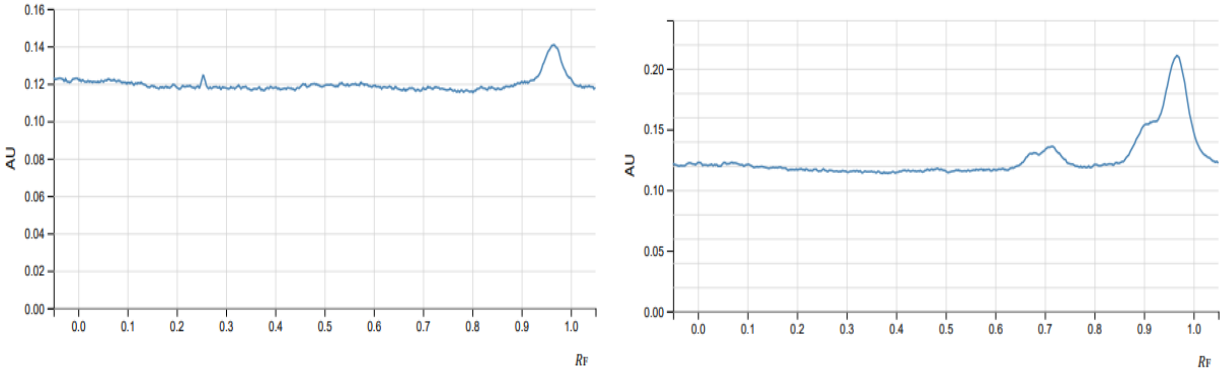


Figure 2. Peaks shown when scanned at 540nm 2.0µl (Track 1) & 5.0µl (Track 2)

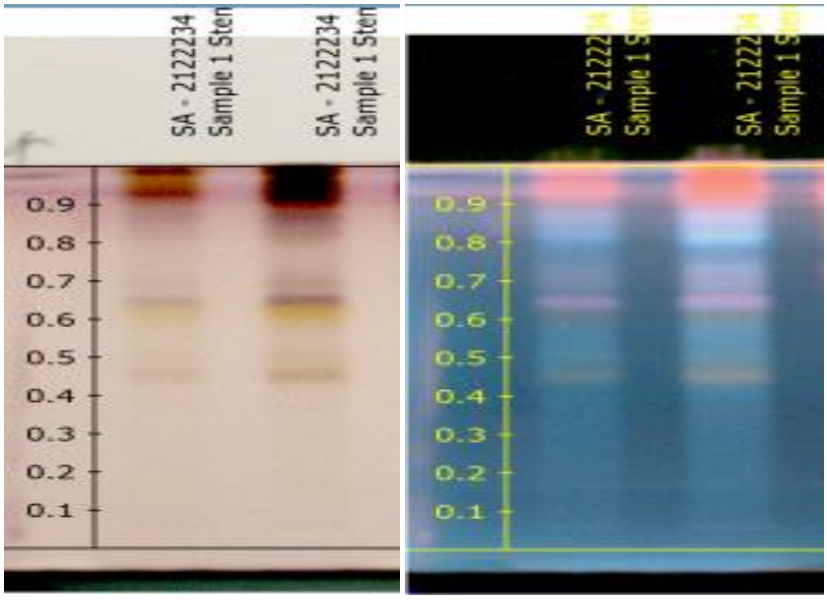
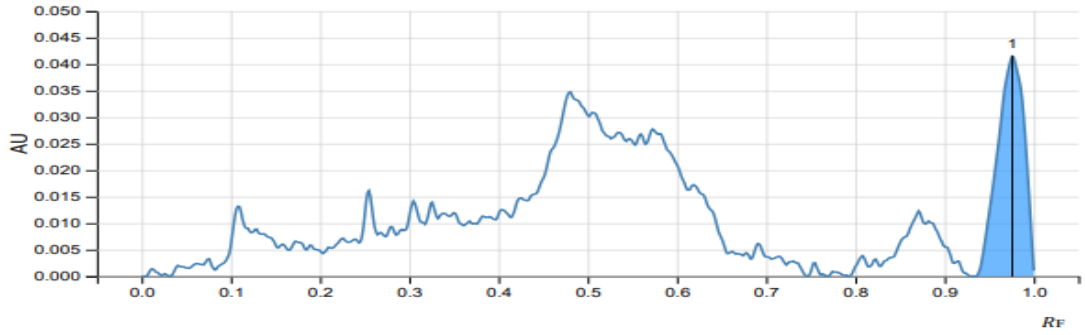
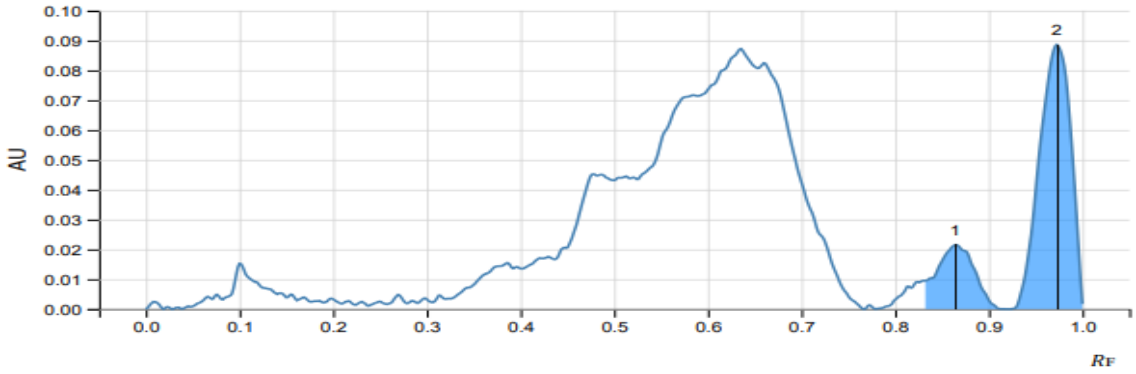


Figure 3. Derivatized Plates showing well differentiated Bands at White & Remission 540nm: Track 1:



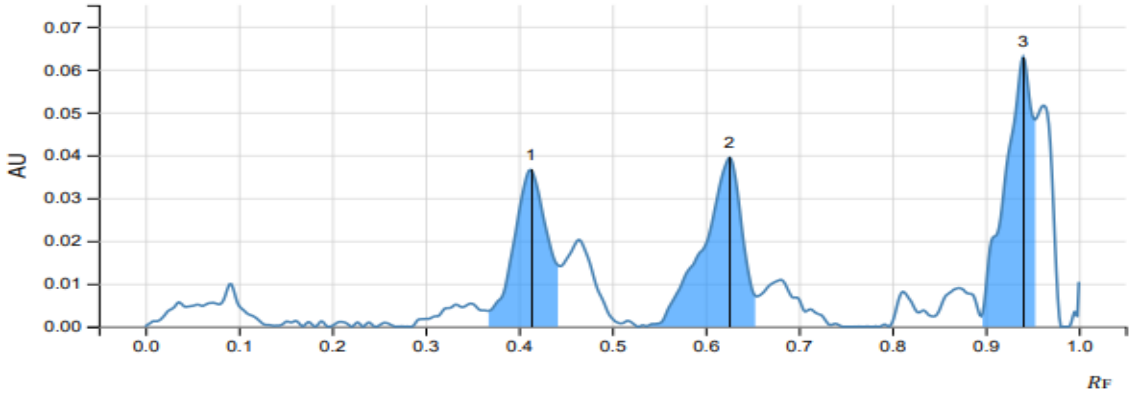
Peak #	Start		Max			End		Area		Manual peak	Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%		
1	0.935	0.0000	0.976	0.0415	100.00	1.000	0.0011	0.00146	100.00	No	

Figure 4. HPTLC showing peak at 540nm at sample Volume 2.0µl after derivatization denotes presence of steroids



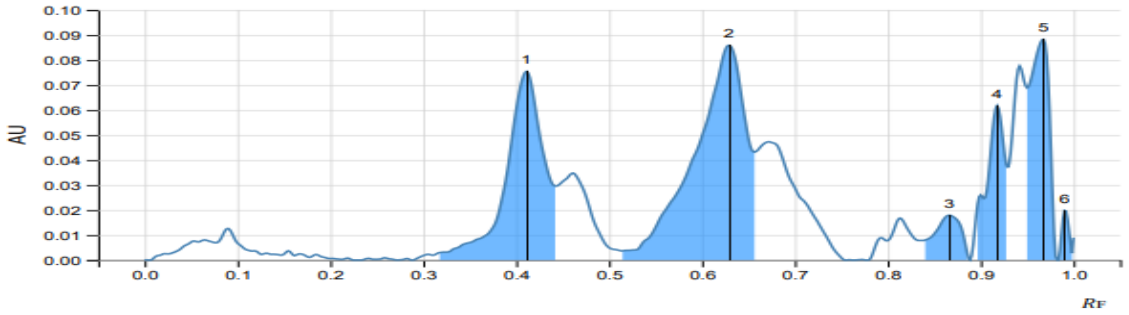
Peak #	Start		Max			End		Area		Manual peak	Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%		
1	0.832	0.0095	0.865	0.0215	19.54	0.915	0.0000	0.00100	22.37	No	
2	0.924	0.0000	0.973	0.0886	80.46	1.000	0.0017	0.00346	77.63	No	

Figure 5. HPTLC showing peak at 540nm at sample Volume 5.0µl after derivatization denotes presence of steroids



Peak #	Start		Max			End		Area		Manual peak	Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%		
1	0.366	0.0037	0.413	0.0366	26.35	0.444	0.0141	0.00153	27.50	No	
2	0.539	0.0002	0.626	0.0394	28.38	0.655	0.0070	0.00192	34.40	No	
3	0.895	0.0025	0.940	0.0629	45.27	0.953	0.0483	0.00212	38.10	No	

Figure 6. HPTLC showing peak at 366nm at sample Volume 2.0µl after derivatization denotes presence of steroids



Peak #	Start		Max			End		Area		Manual peak	Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%		
1	0.316	0.0029	0.411	0.0754	21.62	0.442	0.0296	0.00351	26.66	No	
2	0.513	0.0037	0.629	0.0859	24.61	0.656	0.0432	0.00547	41.60	No	
3	0.839	0.0078	0.866	0.0179	5.14	0.887	0.0006	0.00061	4.60	No	
4	0.895	0.0176	0.918	0.0617	17.67	0.927	0.0384	0.00131	9.95	No	
5	0.950	0.0689	0.968	0.0882	25.28	0.982	0.0000	0.00209	15.89	No	
6	0.984	0.0000	0.990	0.0198	5.68	0.998	0.0027	0.00017	1.30	No	

Figure 7. HPTLC showing peak at 366nm at sample Volume 5.0µl after derivatization denotes presence of steroids

The primary sources of bioactive molecules are natural items, and they have also been crucial in finding the key components for creating drugs to treat human disorders [16]. Isoprenoids, biflavanoids, and alkaloids are three secondary metabolites with bioactive potential that are found in medicinal plants and are particularly important. The entire manufacturing of anticancer chemicals has been prompted by the discovery of anticancer molecules [17–19]. The findings of this study showed that Ipomea sagittifolia leaves contain a variety of steroid 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.

Presence of 2 types of steroids when scanned at 366nm and 6 types of steroids at 540 nm, respectively. The results of the current study showed the presence of 6 different types of steroids. The variation in biochemical levels that can occur has been demonstrated by the HPTLC profile of steroids. The plant's medicinal significance can then be further defined and authenticated using a linear, precise, and accurate HPTLC fingerprinting approach.

In order to determine the presence of various based on plants constituents such as steroids from chromatogram peaks and acquire peak tables, the current study's findings are restricted to the HPTLC examination of an ethanolic extract of an Ipomea sagittifolia stem. Yet, the research continues to require taking quantitative measures of these phytoconstituents.

4. Conclusions

The *Ipomea sagittifolia* plant reportedly contains steroids that are used in conventional medicine to treat a variety of diseases, according to the current study. Isolated substances and their profile are crucial for the creation of medicines. When the Rf values of these substances are compared with standards as a reference, the HPTLC analysis for ethanol-based stem extract of *Ipomea sagittifolia* is helpful in identifying chemical profiles and identification of bioactive components.

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