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HPTLC Fingerprint Analysis Of Ethanolic Leaf Extract of *Ipomoea obscura* (L.) Ker – Gawl

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ABSTRACT

To find out the secondary metabolites present in the ethanolic leaf extract of *Ipomoea obscura* (L.) by means of High Performance Thin Layer Chromatography (HPTLC). Ethanolic extract of the leaves were developed in the mobile phase of formic acid, water, n-hexane-ethyl acetate using standard procedures and scanned under UV at 366nm, 254nm and under visible light. The HPTLC fingerprinting of the ethanolic leaf extract of *Ipomoea obscura* (L.) showed the presence of 7 Flavonoids, 5 Alkaloids, 5 Terpenoids. From this analysis, it has showed that flavonoids are rich in *Ipomoea obscura* (L.). The intensive study on the out coming active constituents of *Ipomoea obscura* (L.) will lead to the discovery of a novel botanical drug.

Keywords: *Ipomoea obscura*, HPTLC, Flavonoid, Fingerprinting profile.

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INTRODUCTION

Many medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparation of the Indian traditional health care system, and proposed for their interesting multilevel activities[1,2]. *Ipomoea obscura* (L.) belongs to the family Convolvulaceae. It is widespread in tropical Africa, South Africa, tropical Asia and northern Australia[3]. Plant grows on fences or low ground cover as substrate in disturbed areas. It is a small climbing vine, with small cordate nutritional, medicinal and ornamental values deserve more research. This plant is effectively used for its curative property in treating dysentery, hemorrhoids and ulcer disorders[4, 5]. Antimicrobial plant extracts represent a continuous effort to find new compounds with the potential to act against multi resistant pathogenic bacteria and fungi[6]. Phytochemical screening of *Ipomoea obscura* (L.) ethanol leaf extraction identified the active phyto chemical constituents. The main objective of this study was to establish a HPTLC finger printing profile of the plant ethanolic leaf extract of *Ipomoea obscura* (L.), which may be used as a marker for quality evaluation and standardization of the drug.

MATERIALS AND METHOD

Plant collection

The whole plant of *Ipomoea obscura* (L) used for the investigation was obtained from Madurai district, Tamil Nadu, India. The plant specimen was authenticated by Dr.G.V.S. Murthy, Botanical Survey of India, TNAU Campus, Coimbatore. The voucher specimen was deposited in the laboratory for future reference (BSI/SRC/5/23/2010-11/Tech). Fresh plant material was washed under running tap water then air dried and powdered.

Extraction

The powder (100g) of plant (Leaf) material was weighed extracted with 500ml of ethanol for 72 hours using occasional shaker. The supernatant was collected and concentrated at 40°C. It was stored at 4°C in an air tight bottle for further use.

HPTLC fingerprinting analysis

The above test solution (2µl) and 2µl of standard solution were loaded as 5mm band length in the 3 x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapour) with respective mobile phases (alkaloid, flavonoid, glycoside, phenol, steroid, tannin and terpenoid) and the plate was developed in the respective mobile phase up to 90mm. The developed plate was dried by hot air to evaporate solvents from the plate. The

plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at Visible light, UV 366nm and UV 254nm. The developed plate was sprayed with respective spray reagent and dried at 100º C in Hot air oven. The plate was photo-documented at Daylight and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) scanning was done at 500nm for terpenoid and 254nm for alkaloid and flavonoid. The peak table, peak display and peak densitogram were noted. The software used was winCATS 1.3.4 version[7].

Mobile Phase

Flavonoid : Toluene – acetone – formic acid(4.5 : 4.5 : 1)

Alkaloid : Ethyl acetate – methanol – water(10 : 1.35 : 1)

Terpenoid : n – Hexane – ethyl acetate (7.2 : 2.9)

Spray Reagent

Flavonoid: 1% Ethanolic aluminium chloride reagent.

Alkaloid: Dragendorff reagent followed with 10% ethanolic sulphuric acid reagent.

Terpenoid: Anisaldehyde sulphuric acid reagent.

RESULTS AND DISCUSSION

Ethnomedicinal plant use data in many forms have been heavily utilized in the development of formularies and pharmacopoeias, providing a major focus in global health care, as well as contributing substantially to the drug development process[8]. The main advantage of using medicinal plant is they does not produce side effects when compared with synthetic drugs, because medicinal plants contain high content of antioxidant compounds[9]. HPTLC is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations. The presence or absence of chemical constituent has been found useful in the placement of the plant in taxonomic categories. HPTLC profile differentiation is such an important and powerful procedure which is often employed for this purpose. The developed HPTLC fingerprints will help the manufacturer for quality control and standardization of herbal formulations[10]. Natural antioxidants which are commonly present in medicinal plants scavenge radicals and inhibit lipid peroxidation and thus prevent oxidative damage in animal tissue or cells[11]. HPTLC fingerprinting of *Ipomoea obscura* (L.) ethanol leaf extract has been shown the 7 flavonoids, 5 alkaloids, 5 terpenoids. The Flavonoids have free radical scavenging and antioxidant properties, which are useful for their pharmacological activities including anticancer and anti-ageing

properties. Flavonoids have important effects on cancer chemoprevention and chemotherapy[12-15]. The Flavonoid compound showed the peak at 2, 3, 4, 6, 7, 8, 12 and eight unknown peak and the Rf value of 0.10, 0.17, 0.22, 0.31, 0.40, 0.51 and 0.70 was shown in the Table 1 and Figure 1 & 2.

Before derivatization **After derivatization**

Day light **UV 366nm** **UV 254nm** **Day light** **UV 366nm**

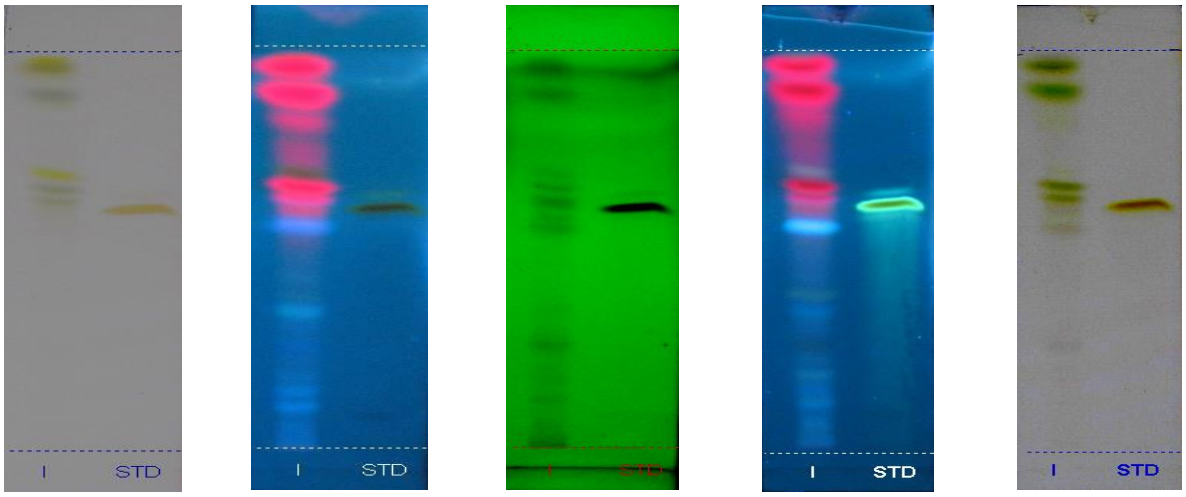
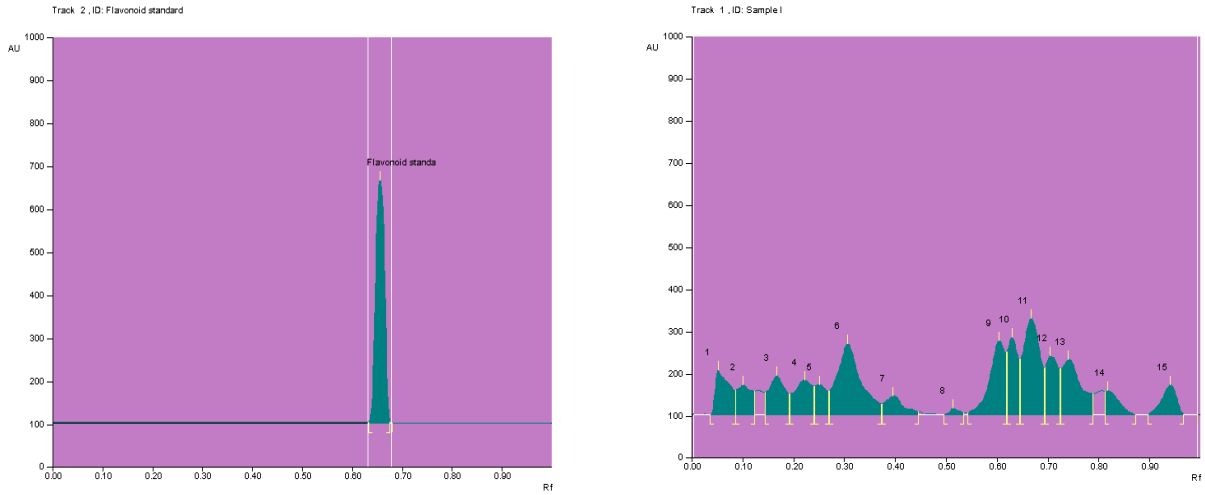


Figure 1: Flavonoid chromatogram before derivatization: Under day light, Under UV 366nm, and 254nm. Chromatogram after derivatization: under day light, Under UV 366nm.



Rutin **Sample**

Figure 2: Peak densitogram display of flavonoid profile of ethanol leaf extract of *Ipomoea obscura* (L.) (Scanned at 254nm)

The alkaloid extracts obtained from medicinal plant species have multiplicity of host-mediated biological activities, antihyperglycemic, anti-inflammatory and pharmacological effects[16,17]. The

alkaloid compound showed the peak at 2, 3, 5, 8, 12 and nine unknown peaks and the Rf value of 0.05, 0.09, 0.24, 0.44 and 0.74 was shown in the Table 2 and Figure 3 & 4.

Table 1: Peak table with Rf values, height and area of flavonoid and unknown compounds in ethanol leaf extract of *Ipomoea obscura* (L.)

Track	Peak	Rf (cm)	Height	Area	Assigned substance
Sample I	1	0.05	106.7	2707.1	Unknown
Sample I	2	0.10	70.6	1879.3	Flavonoid 1
Sample I	3	0.17	92.5	2742.7	Flavonoid 2
Sample I	4	0.22	82.9	2674.0	Flavonoid 3
Sample I	5	0.25	72.1	1513.9	Unknown
Sample I	6	0.31	168.3	7288.2	Flavonoid 4
Sample I	7	0.40	45.5	1482.3	Flavonoid 5
Sample I	8	0.51	16.0	246.4	Flavonoid 6
Sample I	9	0.60	176.4	4941.8	Unknown
Sample I	10	0.63	183.8	3385.2	Unknown
Sample I	11	0.67	228.7	6862.6	Unknown
Sample I	12	0.70	139.8	3148.5	Flavonoid 7
Sample I	13	0.74	131.3	4585.0	Unknown
Sample I	14	0.82	57.1	1446.5	Unknown
Sample I	15	0.94	70.7	1891.7	Unknown
Standard	1	0.73	691.6	19096.9	Rutin

Before derivatization **After derivatization**

Day light **UV 366nm** **UV 254nm** **Day light** **UV 366nm**

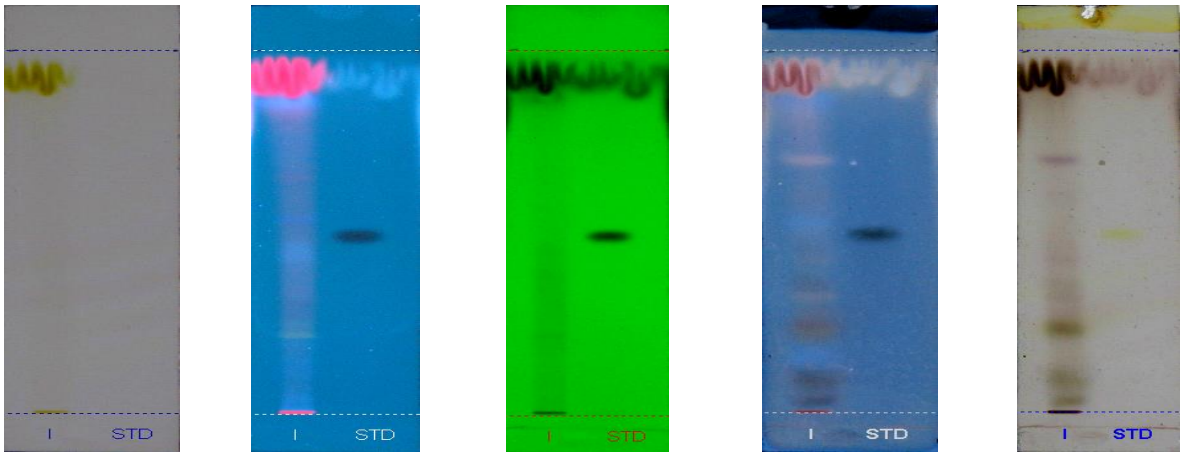
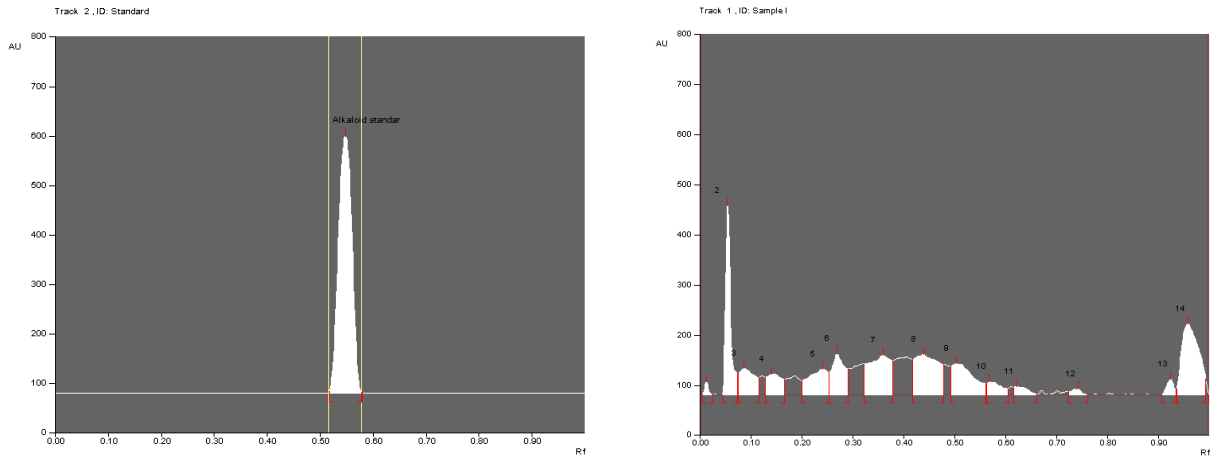


Figure 3: Alkaloid chromatogram before derivatization: Under day light, Under UV 366nm, and 254nm. Chromatogram after derivatization: under day light, Under UV 366nm.



Colchicine **Sample**

Figure 4: Peak densitogram display of alkaloid profile of ethanol leaf extract of *Ipomoea obscura* (L.) (Scanned at 254nm)

Terpenoids have been found to be useful in the prevention and therapy of several diseases. A large number of terpenoids exhibit cytotoxicity against a variety of tumor cells and cancer preventive as well as anticancer efficacy in preclinical animal models[18]. The terpenoid compound showed the peak at 3, 4, 9, 10, 11 and seven unknown peaks and the Rf value of 0.18, 0.23, 0.71, 0.85 and 0.92 was shown in the Table 3 and Figure 5 & 6.

Before derivatization **After derivatization**

Day light **UV 366nm** **UV 254nm** **Day light** **UV 366nm**

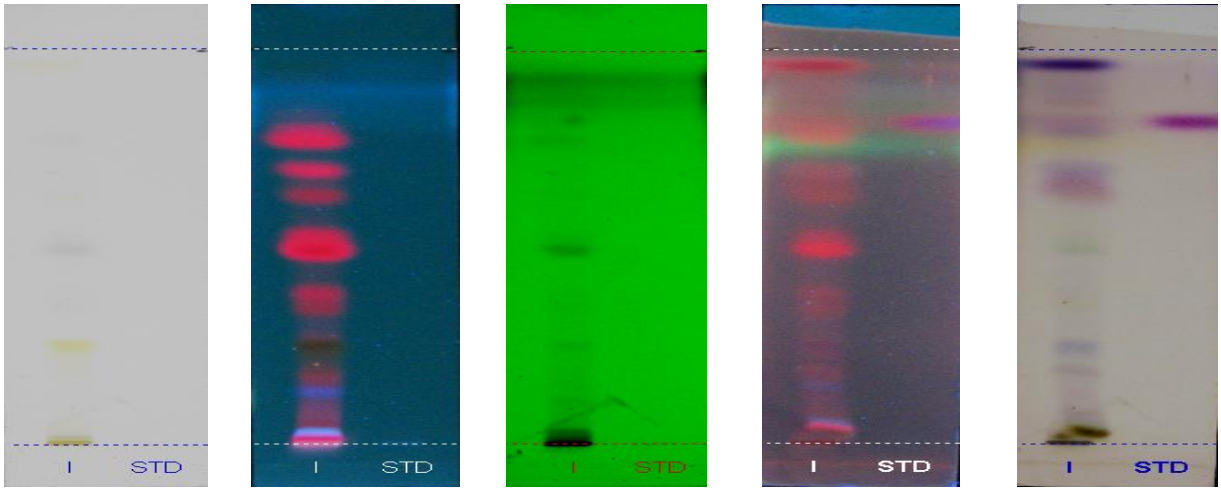
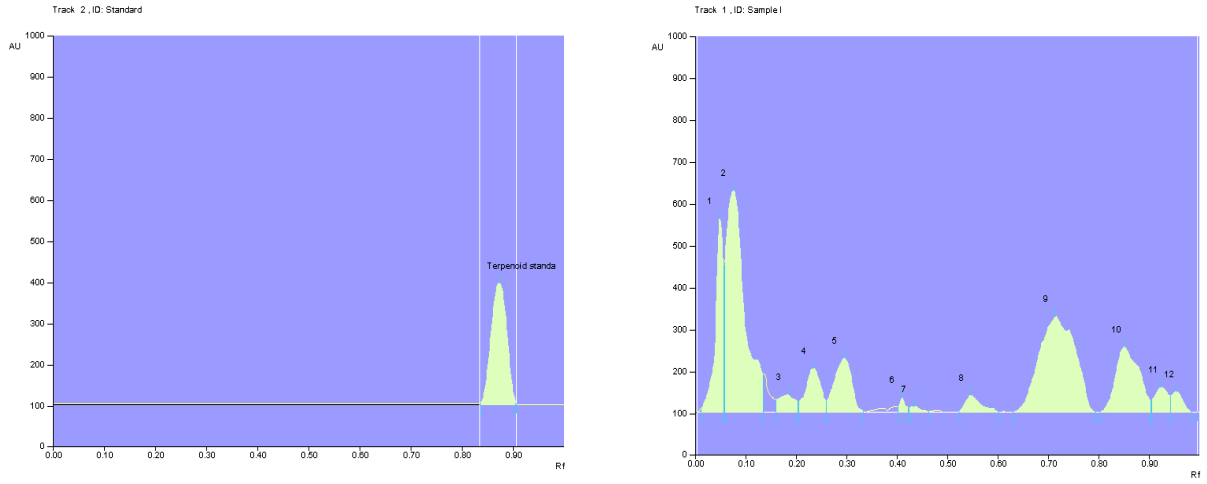


Figure 5: Terpenoid chromatogram before derivatization: Under day light, Under UV 366nm, and 254nm. Chromatogram after derivatization: under day light, Under UV 366nm.



Solanesol **Sample**

Figure 6: Peak densitogram display of terpenoid profile of ethanol leaf extract of *Ipomoea obscura* (L.) (Scanned at 500nm)

Table: 2 Peak table with Rf values, height and area of alkaloid and unknown compounds in ethanol leaf extract of *Ipomoea obscura* (L.)

Track	Peak	Rf (cm)	Height	Area	Assigned substance
Sample I	1	0.01	26.9	174.7	Unknown
Sample I	2	0.05	377.2	3465.9	Alkaloid 1
Sample I	3	0.09	53.1	1472.4	Alkaloid 2
Sample I	4	0.14	41.3	1188.7	Unknown
Sample I	5	0.24	51.1	1787.9	Alkaloid 3
Sample I	6	0.27	81.7	1932.0	Unknown
Sample I	7	0.36	79.3	3252.0	Unknown
Sample I	8	0.44	80.5	3529.0	Alkaloid 4
Sample I	9	0.50	61.8	2471.4	Unknown
Sample I	10	0.57	25.9	674.3	Unknown
Sample I	11	0.62	16.4	404.6	Unknown
Sample I	12	0.74	11.9	204.5	Alkaloid 5
Sample I	13	0.92	39.2	393.5	Unknown
Sample I	14	0.96	141.4	4415.7	Unknown
Standard	1	0.55	539.1	13447.3	Colchicine

Table 3: Peak table with Rf values, height and area of terpenoid and unknown compounds in ethanol leaf extract of *Ipomoea obscura* (L.)

Track	Peak	Rf(cm)	Height	Area	Assigned substance
Sample I	1	0.05	462.7	6502.1	Unknown
Sample I	2	0.07	529.2	18033.6	Unknown
Sample I	3	0.18	42.3	1232.8	Terpenoid 1
Sample I	4	0.23	104.8	2819.0	Terpenoid 2
Sample I	5	0.29	123.4	4043.0	Unknown
Sample I	6	0.41	35.0	346.0	Unknown

Sample I	7	0.43	14.4	272.2	Unknown
Sample I	8	0.55	40.9	1148.1	Unknown
Sample I	9	0.71	228.8	15063.3	Terpenoid 3
Sample I	10	0.85	155.8	6650.1	Terpenoid 4
Sample I	11	0.92	59.3	1459.9	Terpenoid 5
Sample I	12	0.95	49.6	991.4	Unknown
Standard	1	0.87	327.0	10781.1	Solanesol

CONCLUSION

In concluded that the ethanolic extract of *Ipomoea obscura* (L) have a significant amount of secondary metabolites. These metabolites are beneficial for maintenance of human health and chronic degenerative diseases. HPTLC fingerprinting analysis showed the presence of various flavonoids, alkaloids and terpenoids in the ethanolic extract. Among the phytochemical analysis *Ipomoea obscura* (L) ethanolic extract showed the flavonoids are rich, that have been used in folk medicine around the world, especially in China. Flavonoids can be exploited to achieve cancer preventive or therapeutic effects in human. The most active extract could be subjected to isolation of active components and it would be carried out for further pharmacological evaluation.

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