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Phytochemical screening and HPTLC Fingerprint analysis of tuber extracts of *Pueraria tuberosa* (Roxb. ex Willd.) DC., *Ipomoea mauritiana* Jacq. and *Adenia hondala* (Gaertn.) de Wilde

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***Abstract*: The present work analyzed major chemical components present in the methanolic extracts of tubers of *Pueraria tuberosa* (Roxb. ex Willd.) DC. of Fabaceae, *Ipomoea mauritiana* Jacq. of Convolvulaceae and *Adenia hondala* (Gaertn.) de Wilde of Passifloraceae. The tubers were collected from different forest areas of Kerala. Powders of the tubers were extracted successively with HPTLC grade Methanol. Phytochemical screening of the tuber extracts of these plants showed the presence of carbohydrates, glycosides, steroids and flavonoids. HPTLC finger printing of methanolic extract of tuber of *P. tuberosa* revealed 8 peaks with R_f values in the range of 0.21 to 0.84; methanolic extract of *I. mauritiana* showed 5 peaks with R_f values in the range of 0.21 to 0.47 and methanolic extract of *A. hondala* tuber revealed 8 peaks with R_f values in the range of 0.11 to 0.98. HPTLC finger print analysis of the extract demonstrated the presence of possible number of components and the consistent quality of chemical constituents.**

***Keywords*: *Pueraria tuberosa, Ipomoea mauritiana, Adenia hondala,* Phytochemical screening, HPTLC Finger printing, Methanolic extract, R_f values.**

I. INTRODUCTION

Plants are rich sources of bioactive compounds that are useful for the maintenance of health and for the treatment of various diseases. It is now evident that the medicinal value of plants lies in the bioactive phytochemical constituents that induce definite physiological effects on human body. World plant biodiversity is the source of herbal medicines and around 70% population rely on plant based medicines.

Chemical fingerprints obtained by HPTLC are strongly recommended for the purpose of quality control of herbal medicines, since they represent the chemical integrities of herbal medicines and therefore, can be used for authentication and identification of plant products. According to the concept of phytoequivalence, the chromatatographic fingerprints of medicinal plants could be utilized for addressing the issue of quality control in plant based medicines [1].

In view of the above, the HPTLC comparative study of methanolic tuber extacts for polyvalent phytoconstituents and their concentrations in three different plant species namely, *Pueraria tuberosa*, *Ipomoea mauritiana and Adenia hondala* were carried out. Literature study revealed that no such studies were carried out on these three species yet. Pharmacopoeia of India correlates 'Vidari' to the tubers of *Pueraria tuberosa*. It is traditionally used for bleeding disorders, bronchial asthma and urinary disorders and is an ingredient of ayurvedic product Chyavanaprasam [2]. Apart from this *Ipomoea mauritiana* and *Adenia hondala* are also traded as 'Vidari'. These substitutes of Vidari may or may not resemble *P.*

tuberosa in terms of morphology, phytochemistry and pharmacology. No reports are available for the comparative phytochemical fingerprinting, so the present study was designed to study the phytochemical constituents of all three different plant drugs under the same local name of 'Vidari'.

II. MATERIALS AND METHODS

2.1 Collection and identification of plant materials:

The plant tubers for the proposed study were collected from different forest areas of Kerala (*P. tuberosa* from Nelliampathy, *I. mauritiana* from Desamangalam and *A hondala* from Wyanad). The tubers were authenticated by Dr. P. S. Udayan, Sree Krishna College, Guruvayur and the voucher specimens were preserved for further reference.

2.2 Preparation and extraction of Plant material:

25 gms of coarsely powdered tubers of *P. tuberosa, I. mauritiana and A. hondala* were taken separately in conical flasks and extracted with 250 ml of methanol using a magnetic stirrer for 12 hours. The process was repeated and the extract was filtered through Whatmann filter paper (no.1).The fraction was evaporated and stored at 8^0 C in air-tight bottles until further use.

2.3 Phytochemical screening:

The preliminary phytochemical analysis of the methanolic tuber extracts of *P. tuberosa, I. mauritiana and A. hondala* was performed with standard protocol [3].

2.4 HPTLC (High performance Thin Layer Chromatography):

2.4.1 Sample preparation

Each extract residue was re-dissolved in 1 ml of chromatographic grade methanol, which was used for sample application on precoated silica gel 60F 254 HPTLC plates of 0.2 mm thickness.

2.4.2 Developing solvent system

The mobile phase composition was optimized by testing various solvent systems and a satisfactory resolution was obtained in the solvent, Toluene: ethyl acetate: formic acid (5:4:0.1).

2.4.3 Application of sample

Samples were applied to the precoated silica gel 60F 254 HPTLC plates of 20x20 cm thickness using Linomat 5 applicator attached to CAMAG HPTLC system, which was equipped with WINCATS software.

2.4.4 Development of chromatogram

After the application of sample, the plates were eluted in pre-saturated twin trough glass chamber 10x 10 cm at room temperature with the mobile phase Toluene: ethyl acetate: formic acid (5: 4: 0.1).

2.4.5 Detection of spots

The air dried plates were viewed in UV radiation to mid day light. The spots were visualized under CAMAG UV cabinet 254 and 366 nm. Then the chromatograms were scanned in the densitometer using CAMAG TLC scanner III. The finger print data and the R_f values were recorded by WINSCATS software.

III. RESULTS

The preliminary phytochemical analysis of methanolic extract of *P. tuberosa, I. mauritiana* and *A. hondala* showed the presence of different phytoconstituents like carbohydrates, glycosides, steroids, and flavonoids (Table1, Figure1). Resin, Tannin, gum and fat were found to be absent in of all the three samples where as alkaloids, saponins and phenols were absent in *A. hondala*. (Table1). The HPTLC fingerprint results scanned at wavelength 550 nm for methanolic extract of *P. tuberosa* tubers showed the presence of 8 polyvalent phytoconstituents and the corresponding order of R_f values start from 0.21 to 0.84 in which the highest concentration of phytoconstituents was found to be 5.52% and its corresponding R_f value was 0.21 and was recorded in table 2. The corresponding HPTLC chromatogram was presented in figure 2.

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The HPTLC fingerprint results scanned at wavelength 550 nm for methanolic extract of *I. mauritiana* tubers showed the presence of 5 polyvalent phytoconstituents and the corresponding ascending order of R_f values start from 0.21 to 0.47. The highest concentration of phytoconstituents was found to be 7.28% and its corresponding R_f value was 0.34 and was recorded in table 3. The corresponding HPTLC chromatogram was presented in figure 3. The R_f values, 0.21 and 0.31are shared by both *P. tuberosa* and *I. mauritiana*.

The HPTLC fingerprint results scanned at wavelength 550 nm for methanolic extract of *A. hondala* tubers showed the presence of 7 polyvalent phytoconstituents. The corresponding order of R_f values start from 0.11 to 0.98 and are unique to *A. hondala* only. The highest concentration of phytoconstituents was found to be 17.90 % and its corresponding R_f value was 0.11 and was recorded in table 4. The corresponding HPTLC chromatogram was presented in figure 4.

TABLE I: Phytochemical Screening of methanolic extracts of *P.tuberosa, I.mauritiana* and *A. hondala*

Constituents	Test	*P. tuberosa*	*I.mauritiana*	*A.hondala*
Sugar &Carbohydrate	Molisch's test	+	+	+
	Fehling's test	+	+	+
	Benedict's test	+	+	+
Alkaloids	Meyer's test	+	+	-
	Dragendorff's reagent	+	+	-
	Wagner's reagent	+	+	-
Glycosides	Keller-Killani test	+	+	+
	Borntrager's test	+	+	+
	Legal's test	+	+	+
Saponin	Foam test	+	+	-
Fat	Spot test	-	-	-
	Saponification test	-	-	-
Steroids	Liebermann-Burchard test	+	+	+
	Salkowski reaction	+	+	+
	Libermann's test	+	+	+
Resin	Turbidity test	-	-	-
	Hydrochloric acid test	-	-	-
Tannin	Ferric chloride test	-	-	-
	Lead acetate test	-	-	-
Phenols	Ferric chloride test	+	+	-
	Lead acetate test	+	+	-
	Gelatin solution	+	+	-
Flavonoids	Shinoda test	+	+	+
Gums	Swelling test	-	-	-

+ Present, - Absent

TABLE II: HPTLC profile of methanolic extract of *Pueraria tuberosa* tuber at 550 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.10 Rf	2.4 AU	-0.09 Rf	89.0 AU	9.03 %	-0.08 Rf	74.0 AU	1185.5 AU	4.73 %
2	-0.05 Rf	152.7 AU	0.00 Rf	640.1 AU	64.99 %	0.06 Rf	2.6 AU	19109.6 AU	76.26 %
3	0.19 Rf	3.2 AU	0.21 Rf	54.4 AU	5.52 %	0.24 Rf	7.6 AU	887.8 AU	3.54 %
4	0.24 Rf	8.0 AU	0.27 Rf	50.2 AU	5.10 %	0.29 Rf	14.3 AU	944.6 AU	3.77 %
5	0.29 Rf	14.5 AU	0.31 Rf	35.4 AU	3.59 %	0.32 Rf	16.6 AU	446.6 AU	1.78 %
6	0.32 Rf	16.7 AU	0.35 Rf	47.3 AU	4.80 %	0.38 Rf	6.2 AU	947.3 AU	3.78 %
7	0.50 Rf	0.1 AU	0.51 Rf	17.0 AU	1.72 %	0.52 Rf	13.3 AU	163.5 AU	0.65 %
8	0.80 Rf	7.8 AU	0.84 Rf	51.5 AU	5.23 %	0.87 Rf	5.2 AU	1374.1 AU	5.48 %

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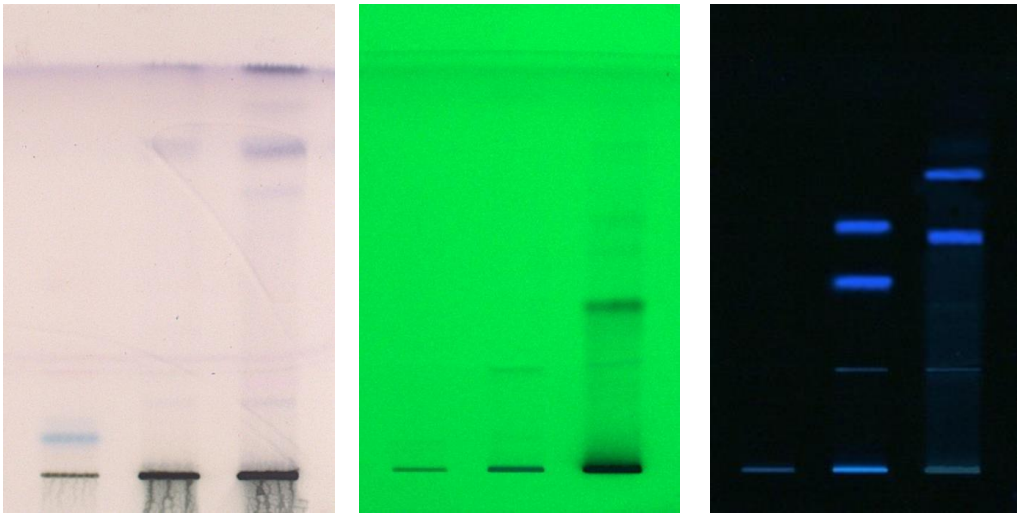
TABLE III: HPTLC profile of methanolic extract of *Ipomoea mauritiana* tuber at 550 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.06 Rf	171.1 AU	0.00 Rf	712.2 AU	81.64 %	0.06 Rf	0.3 AU	21484.1 AU	88.54 %
2	0.18 Rf	0.0 AU	0.21 Rf	26.7 AU	3.06 %	0.24 Rf	1.3 AU	466.2 AU	1.92 %
3	0.27 Rf	3.5 AU	0.31 Rf	39.4 AU	4.51 %	0.32 Rf	35.5 AU	652.8 AU	2.69 %
4	0.32 Rf	35.7 AU	0.34 Rf	63.5 AU	7.28 %	0.38 Rf	10.1 AU	1386.7 AU	5.71 %
5	0.46 Rf	4.3 AU	0.47 Rf	30.6 AU	3.51 %	0.49 Rf	3.2 AU	274.1 AU	1.13 %

TABLE IV: HPTLC profile of methanolic extract *Adenia hondala* tuber at 550 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.07 Rf	26.5 AU	0.00 Rf	602.0 AU	62.75 %	0.04 Rf	21.3 AU	8757.4 AU	48.84 %
2	0.07 Rf	28.8 AU	0.11 Rf	171.7 AU	17.90 %	0.20 Rf	7.3 AU	5373.7 AU	29.97 %
3	0.26 Rf	1.5 AU	0.31 Rf	57.1 AU	5.95 %	0.33 Rf	23.9 AU	1056.3 AU	5.89 %
4	0.33 Rf	24.6 AU	0.35 Rf	61.9 AU	6.46 %	0.40 Rf	6.5 AU	1380.3 AU	7.70 %
5	0.40 Rf	6.9 AU	0.42 Rf	26.7 AU	2.79 %	0.46 Rf	6.3 AU	488.8 AU	2.73 %
6	0.53 Rf	6.6 AU	0.57 Rf	15.4 AU	1.60 %	0.60 Rf	10.4 AU	487.4 AU	2.72 %
7	0.60 Rf	11.0 AU	0.61 Rf	11.1 AU	1.15 %	0.65 Rf	3.4 AU	300.7 AU	1.68 %
8	0.96 Rf	2.1 AU	0.98 Rf	13.4 AU	1.40 %	0.99 Rf	0.8 AU	87.1 AU	0.49 %



HPTLC plate seen at visible HPTLC plate seen at 254nm HPTLC plate seen at 366nm light

Track 1: *Adenia hondala*

Track 2: *Ipomoea mauritiana*

Track 3: *Pueraria tuberosa*

Fig 1: HPTLC profile of tuber extract of *P. tuberosa, I. mauritiana* and *A. hondala*

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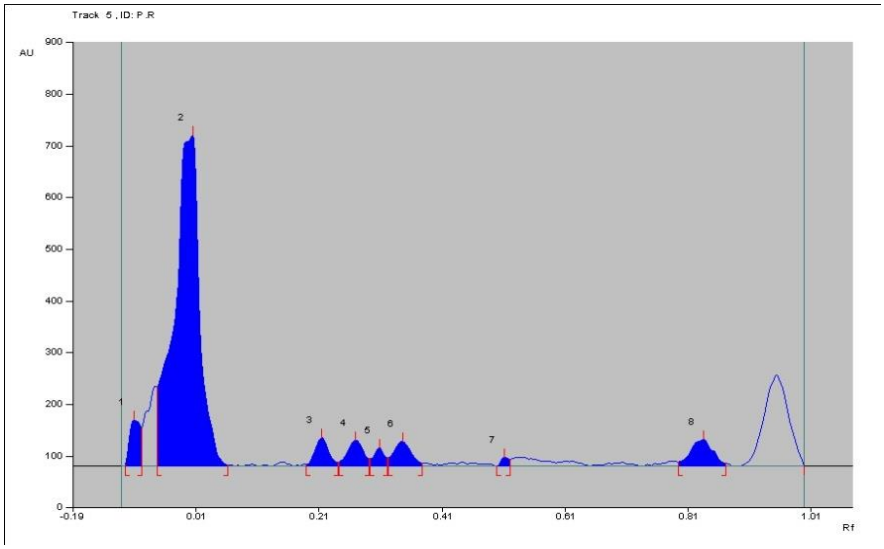

Fig 2: HPTLC Chromatogram of *Pueraria tuberosa* methanolic tuber extracts showing different peaks of phytoconstituents at 550nm

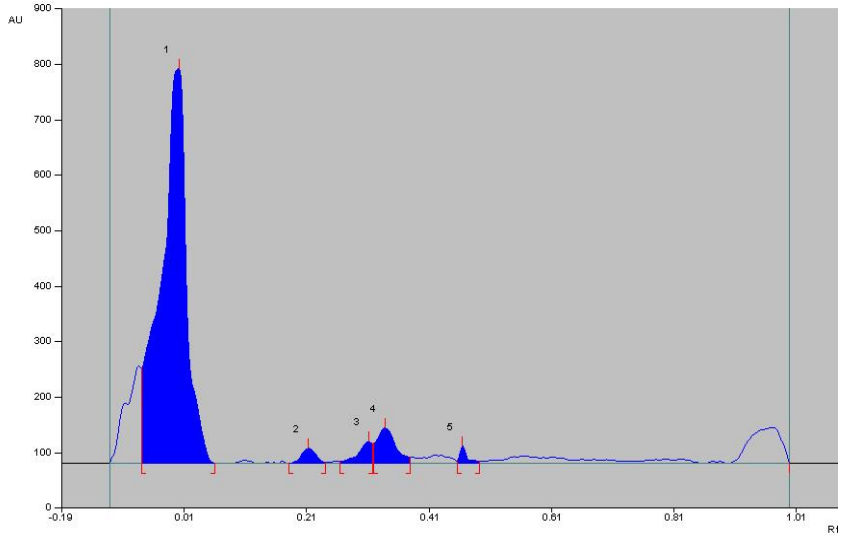

Fig 3: HPTLC Chromatogram of *Ipomoea mauritiana* methanolic tuber extracts showing different peaks of phytoconstituents at 550 nm

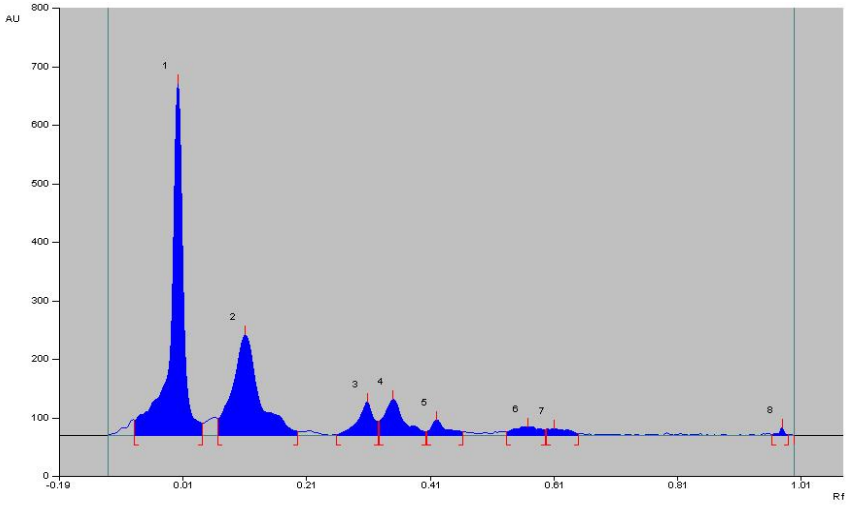

Fig 4: HPTLC Chromatogram of *Adenia hondala* methanolic tuber extracts showing different peaks of phytoconstituents at 550nm

IV. DISCUSSION

4.1 Phytochemical screening:

Plant based bioactive constituents are attractive candidates for drug industry. The demand of medicinal plants has increased tremendously, hence there is a great need for a simple and rapid analytical methods for the manufacture of plant-derived medicines. Particular plant can be identified and distinguished from its closely related species through HPTLC fingerprinting. The phytochemical test on methanolic extracts of *P. tuberosa*, *I. mauritiana* and *A. hondala* showed the presence of various phytochemicals like carbohydrates, glycosides, saponins, phytosterols, phenols and flavonoids where as resins, fats and tannins were absent in all 'vidari' species. Qualitative screening of phytochemicals for *P. tuberosa and I. mauritiana* indicated the presence of alkaloids whereas carbohydrates, glycosides, saponins, phytosterols, phenols, gums, flavonoids and proteins are the various phytoconstituents reported in all the 'Vidari' species [4].

Various types of secondary metabolites are produced by plants, that have been subsequently utilized by humans in a different array of applications. The glycosides contribute to almost every therapeutic class, renal disinfectants, antirheumatics, anti-inflammatory, cardiac drugs, expectorant, laxatives, analgesics and antispasmodic action [5]. Steroids are used for the treatment of allergic reactions, arthritis and for various diseases resulting from hormone deficiencies [6]. Terpenoids are the secondary metabolites that show significant pharmacological activities, like antibacterial, antiviral, anti-inflammatory, antimalarial and anticancer activities [7], [8]. Phytosterols, triterpenes, flavonoids, tannins, glycosides, saponins, alkaloids and carbohydrates were the different phytoconstituents present in the ethanolic, methanolic and ethyl acetate extract of *Coccinia cordifolia* [9].

4.2 HPTLC fingerprinting:

The HPTLC fingerprints of three varieties of Romanian white wines (Sauvignon Blanc, Riesling, and Feteasca Alba) are beneficial for quality control of wines and the differentiation of white wines in terms of their variety [10.]. Distinct phytochemical as well as microscopic characters of *I. mauritiana* tubers have been developed for quality control of crude drugs [11]. The Total Phenolic Content (TPC) was found to be highest in acetone fraction whereas it was lowest in chloroform fraction of *I. mauritiana* [12].

Ethanolic and aqueous extract of *P. tuberosa* tubers revealed prominent cytotoxicity whereas, poor cytotoxicity was exhibited by Soxhlet apparatus-extracted butanolic extract and microwave-assisted butanolic extracts [13]. HPTLC chromatogram of fingerprint analysis of ethanolic extract of *P. tuberosa* using chloroform and methanol (9:1), showed eleven compounds with R_f values ranging from 0.00 to 0.71 [14].

V. CONCLUSION

The HPTLC fingerprints of *P. tuberosa, I. mauritiana* and *A. hondala* will help the manufacturer to distinguish the adulterant. Such chemo finger printing will definitely act as biochemical markers for these three plant species and will help the standardization of herbal formulations in pharma industry.

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