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Development of pharmacopoeial standards and HPTLC chromatographic finger print analysis of the roots of *Hibiscus vitifolius* Linn

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Abstract

Herbal medicines have a long therapeutic history and are still serving many of the health needs of a large population of the world. However, the quality control and quality assurance still remains a challenge because of the high variability of chemical components involved. Herbal drugs, singularly and in combinations contains numerous active compounds responsible for the overall efficacy. This creates a challenge in establishing the standardization of herbal drugs and finished products. *Hibiscus vitifolius* Linn., family Malvaceae, is widely distributed in tropical and subtropical regions of the world. The roots of the plant are demulcent, contraceptive and used in the treatment of pulsating anterior fontanelle in babies and kidney problems. In view of its medicinal importance it becomes necessary to evaluate the physic-chemical parameters, preliminary phytochemical analysis and TLC/HPTLC finger print analysis of n-hexane, chloroform, ethyl acetate and ethanol extracts. TLC and HPTLC finger print profiles for the various extracts of *H. vitifolius* can be used as a quality control tool. These profiles can be used for rapid identification, monitoring the purity and detection of adulterants. This will help in confirming the genuinity of the material.

Keywords: *Hibiscus vitifolius* Linn., Physico-chemical parameters, Preliminary phytochemical analysis, TLC/HPTLC finger print profile.

INTRODUCTION

India has a rich heritage of traditional medicine like Ayurveda, Siddha, Unani, Homoeopathy and Naturopathy. Traditional health care has been flourishing in this country for many centuries[1]. Natural remedies from medicinal plants are found to be safe and effective. Many plants species have been used in folkloric medicine to treat various ailments[2].

The method HPTLC is useful in describing the identification, standardization and quantification of active phytoconstituents in the plant, plant extract and their formulations. The safety concern has become necessary issue to standardize these herbal medicines by using TLC and HPTLC finger print. The standardization of raw materials in herbal industry is an important step towards quality control.

The genus *Hibiscus* (Malvaceae) consists of 200 species, widely distributed in the tropical subtropical region of the world. There are about 40 species grown in India. Many *Hibiscus* species are valued as ornamental plants and cultivated in gardens. Some species are used for medicinal properties. *Hibiscus vitifolius* Linn., syn. *Fioria vitifolia* (L.) Mattei is mentioned as wild okra in English. The roots are demulcent and are used as contraceptive. They are also used in the treatment of pulsating anterior fontanelle in babies, in kidney problems. The seeds are considered as stimulant and anti-spasmodic. Aqueous extracts of root showed hepatotoxicity activity[3,4,5]. The present work is to frame the preliminary phytochemical analysis and HPTLC finger print analysis of n-hexane, chloroform, ethyl acetate and ethanol extracts.

MATERIALS AND METHODS

The fresh roots of *Hibiscus vitifolius* Linn. was collected from Chennai, Tamil Nadu, India. It was authenticated by Dr. P. Jayaraman, Plant Anatomy Research Centre, West Tambaram, Chennai, India. A voucher specimen (No. 00627) of the roots has been deposited in the herbarium of Regional Research

Institute of Unani Medicine (CCRUM), Chennai, Tamil Nadu, India. The collected roots were shade dried, powdered and the powders were used for the studies.

Physicochemical parameters

Physico-chemical parameters like total ash, acid insoluble ash, alcohol and water soluble extractives, successive extractive values and loss on drying at 105 °C methods were carried out as per WHO guidelines[6].

Preparation of the extracts

The coarse powder of dried root (5 g) of the *H. vitifolius* was successively extracted with n-hexane, chloroform, ethyl acetate and ethanol using Soxhlet apparatus. The extracts were made up to 10 ml in a standard flask with respective solvents separately[7,8,9].

Preliminary phytochemical screening

The n-hexane, chloroform, ethyl acetate and ethanol extracts were subjected to preliminary phytochemical analysis to find out the various classes of organic compounds present in the extracts by using standard methods[10,11,12].

Solvents and reagent

All solvents and reagents used in the study were of Analytical grade[13,14].

TLC/HPTLC finger print studies

Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) were performed as per the standard methods[15,16].

Chromatographic conditions

CAMAG HPTLC instrument, sample applicator - CAMAG Linomat - IV applicator with N2 gas flow, photodocumentation system - Digi store - 2 documentation system with Win Cats & video scan software, scanner - CAMAG HPTLC scanner - 3 (030618), Win Cats - IV, development chamber - CAMAG TLC 10x10, 10x20 twin trough linear development chamber, quantity applied - 5, 10 µl of extracts, stationary phase - Aluminium plate pre-coated with silica gel 60 F254 (E. Merck), plate thickness - 0.2 mm, scanning wavelength - 254 nm, laboratory condition - 26 ± 5^{o}C and 53 % relative humidity.

Developing solvent system

The developing solvent system for the n-hexane extract - toluene: ethyl acetate (9:1); chloroform extract - toluene: ethyl acetate (3:1); ethyl acetate extract - chloroform: methanol: formic acid (9:1: 0.2) and ethanol extract - ethyl acetate: methanol: formic acid (9:1: 0.2) were used.

Spray reagent: Vanillin – Sulphuric acid

Vanillin (1g) was dissolved in 95 ml of ice cold alcohol, 5 ml of 36 N sulphuric acid cooled in ice was added and stirred well. The reagent was kept in a refrigerator.

Development of TLC and HPTLC

The plate was developed upto a height of 8 cm, air dried, spots were observed under the UV light at 254 nm and 366 nm. The plates were scanned at 254 nm and finger print chromatogram was recorded. Finally the plates were derivatized using vanillin-sulphuric acid reagent and heated at 105° C till colour spots appeared.

RESULTS

Physico-chemical parameters like foreign matter, loss on drying at 105º C, ash values, extractive values are given in table 1. These data can be used for identification of the drug. Quantitative standards revealed that the ash content was 5.36 % which states that negligible amount of acid insoluble siliceous matter was detected in the drug. The water soluble ash content of the drug indicated the presence of inorganic content. The alcohol soluble extractive value 3.69 % is the indication the presence of polar and non polar secondary metabolities present in the drug. Water soluble extractive value indicates 9.67 % the presence of sugar, acids and inorganic components in the sample.

Table 1: Physico-chemical parameters of the root powder of *H. vitifolius*

S. No	Parameters	Results (n = 3) ± SD
1.	% Foreign matter	Nil
2.	% Loss on drying at 105ºC	10.75 ± 0.07
3.	% Ash	5.36 ± 0.01
4.	% Water soluble ash	2.43 ± 0.03
5.	% Acid insoluble ash	0.09 ± 0.05
6.	% Solubility at room temp.	
	a. Ethanol	3.69 ± 0.12
	b. Water	9.67 ± 0.09
7.	% Successive extractive values:	
	a. n-hexane	1.45 ± 0.06
	b. Chloroform	1.12 ± 0.03
	c. Ethyl acetate	1.09 ± 0.02
	d. Ethanol	4.73 ± 0.04

Phytochemical screening of *H. vitifolius* root showed the presence of alkaloids, amino acids, flavonoids, phenols, tannins, glycosides/sugars, steroids, triterpenoids, saponins and coumarins in n-hexane, chloroform, ethyl acetate and alcohol extracts (Table 2).

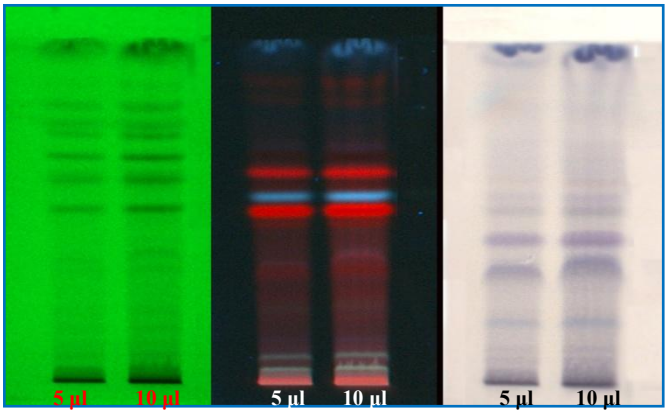
Table 2: Preliminary phytochemical tests of the root of *H. vitifolius*

S. No	Test	n-hexane	Chloroform	Ethyl acetate	Ethanol
1.	Alkaloid	-	+	+	+
2.	Amino acid	-	-	-	+
3.	Coumarin	-	+	-	-
4.	Flavonoid	-	+	+	+
5.	Glycoside/Sugar	-	-	+	+
6.	Phenol	-	-	+	+
7.	Quinone	-	-	-	-
8.	Steroid	+	+	+	+
9.	Tannin	-	-	+	+
10.	Triterpenoid	-	+	+	-
11.	Saponin	-	+	+	+
12.	Furanoid	-	-	-	-
13.	Lignan	-	-	-	-
15.	Carboxylic acid	-	-	-	-
+ = Present			- = Absent		

TLC profile of n-hexane extract of the roots of *H. vitifolius*

The TLC profile of n-hexane extract is shown in Fig. 1. The R_f values of various spots in the TLC profile are given in the Table 3. At 254 nm 12

spots and 366 nm 11 spots were observed. After derivatisation with vanillin-sulphuric acid 9 spots were observed.



UV- 254 nm UV – 366 nm Visible light (After derivatization with V-S reagent)

Fig. 1: TLC of n-hexane extract of *H. vitifolius* Linn.

Table 3: TLC profile of n-hexane extract of the roots of *H. vitifolius*

Solvent System	Rf Values		
	UV - 254 nm	**UV - 366 nm**	**Visible light (after derivatisation with Vanillin – Sulphuric acid)**
Toluene: Ethyl acetate (9:1)	0.83 Green	0.85 Red	0.83 Grey
	0.78 Green	0.80 Red	0.70 Grey
	0.74 Green	0.67 Violet	0.58 Pink
	0.71 Green	0.62 Red	0.54 Violet
	0.65 Green	0.52 Fluorescent blue	0.50 Grey
	0.58 Green	0.49 Red	0.41 Violet
	0.50 Green	0.44 Red	0.33 Violet
	0.37 Green	0.36 Pink	0.18 Blue
	0.31 Green	0.31 Red	0.10 Grey
	0.20 Green	0.18 Red	
	0.15 Green	0.10 Fluorescent blue	
	0.12 Green		

HPTLC finger print profile of n-hexane extract of the roots of *H. vitifolius*

HPTLC finger print profile of n-hexane extract showed 14 peaks (Fig. 2) of which 7 were major peaks at R_f 0.45, 0.54, 0.61, 0.68, 0.71, 0.77 and 0.91 whereas peaks at R_f 0.02, 0.07, 0.12, 0.21, 0.26, 0.32 and 0.83 were moderately smaller peaks.

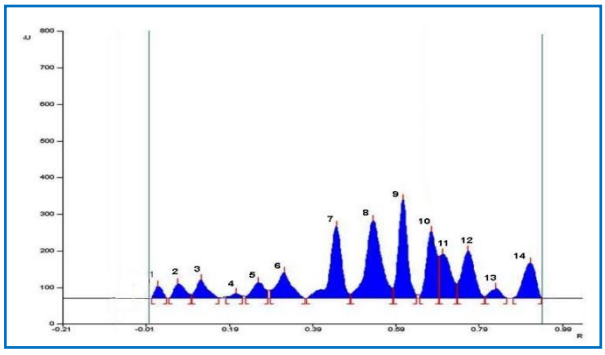
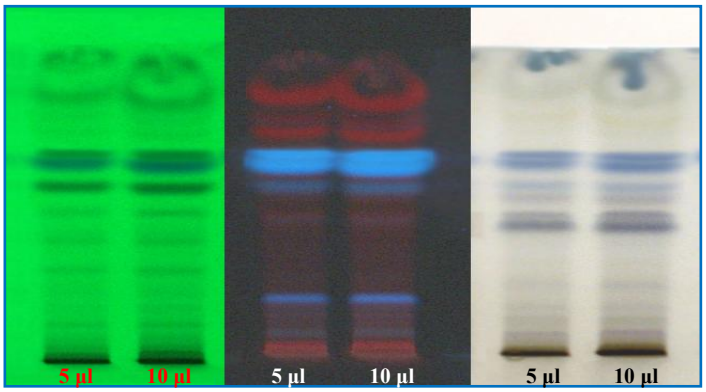


Fig. 2: HPTLC finger print profile of the n-hexane extract of the roots *of H. vitifolius*

TLC profile of chloroform extract of the roots of *H. vitifolius*

TLC profile of chloroform extract is shown in Fig. 3. The R_f values of various spots in the TLC profile are given in the Table 4. At 254 nm 11 spots and 366 nm 9 spots were observed. The plate when derivatized with vanillin-sulphuric acid showed 11 spots.



UV- 254 nm UV – 366 nm Visible light (After derivatization with V-S reagent)

Fig. 3: TLC of chloroform extract of the roots of *H.vitifolius*

Table 4: TLC profile of chloroform extract of the roots of H. vitifolius

Solvent System	Rf Values		
	UV - 254 nm	**UV - 366 nm**	**Visible light (after derivatisation with Vanillin – Sulphuric acid)**
Toluene: Ethyl acetate (3:1)	0.82 Green	0.83 Red	0.83 Blue
	0.75 Green	0.77 Red	0.73 Yellow
	0.72 Green	0.72 Red	0.65 Blue
	0.65 Green	0.65 Fluorescent blue	0.61 Blue
	0.61 Green	0.61 Fluorescent blue	0.54 Blue
	0.54 Green	0.57 Red	0.49 Violet
	0.46 Green	0.54 Blue	0.44 Violet
	0.37 Green	0.46 Red	0.41 Violet
	0.28 Green	0.19 Blue	0.21 Grey
	0.21 Green		0.15 Violet
	0.10 Green		0.11 Grey

HPTLC finger print profile of chloroform extract of the roots of *H. vitifolius*

HPTLC finger print profile of chloroform extract showed 15 peaks (Fig. 4) of which 4 were major peaks at R_f 0.54, 0.61, 0.65 and 0.91 whereas peaks at R_f 0.03, 0.08, 0.13, 0.19, 0.23, 0.27, 0.32, 0.39, 0.45, 0.80 and 0.99 were moderately smaller peaks.

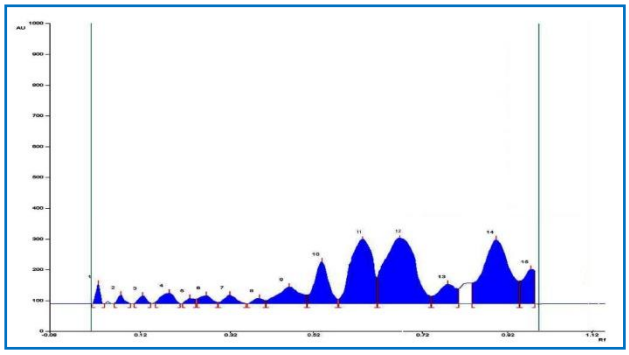
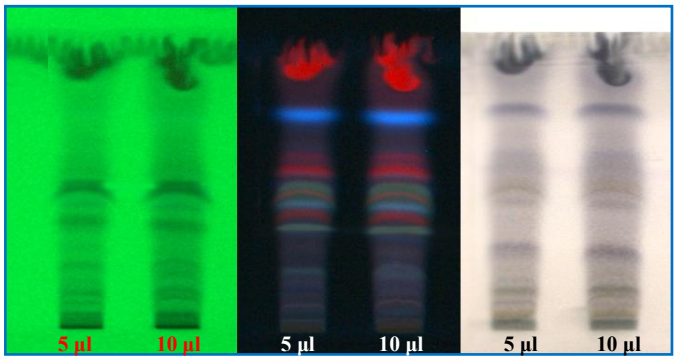


Fig. 4: HPTLC finger print profile of the chloroform extract of the roots of *H. vitifolius*

TLC profile of ethyl acetate extract of the roots of *H. vitifolius*

The TLC profile of ethyl acetate extract is shown in Fig. 5. The *Rf* values of various spots in the TLC profile are given in the Table 5. At 254 nm 10 spots and 366 nm 12 spots were observed. Derivatization with vanillin-sulphuric acid reagent showed 10 spots.



UV- 254 nm UV – 366 nm Visible light (After derivatization with V-S reagent)

Fig. 5: TLC of ethyl acetate extract of the roots of *H. vitifolius*

HPTLC finger print profile of ethyl acetate extract

HPTLC finger print profile of ethyl acetate extract showed 13 peaks (Fig. 6) of which 3 were major peaks at R_f 0.31, 0.44 and 0.92 whereas peaks at R_f 0.03, 0.08 0.11, 0.20, 0.34, 0.47, 0.52, 0.57, 0.65, 0.77 and 0.82 are moderately smaller peaks.

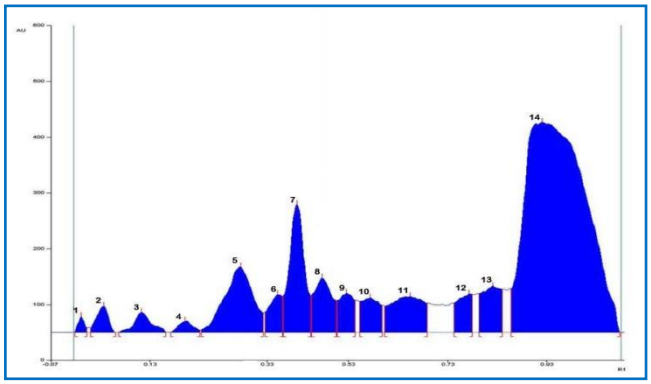


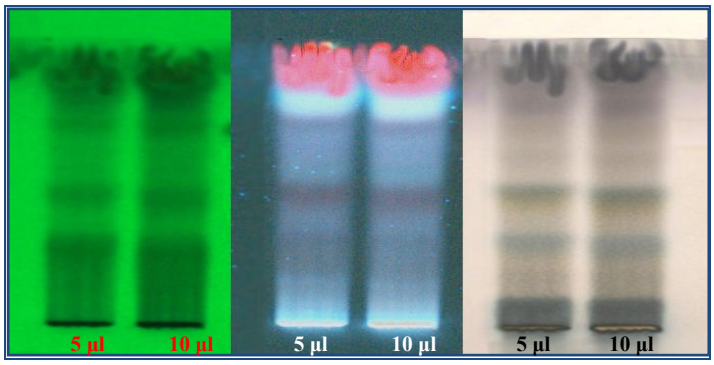
Fig. 6: HPTLC finger print profile of the ethyl acetate extract of the roots *of H. vitifolius*

TLC profile of ethanol extract of the roots of *H. vitifolius*

The TLC profile of ethanol extract is shown in Fig. 7. The *Rf* values of various spots in the TLC profile are given in the Table 6. At 254 nm and 366 nm 8 spots were observed. The plate when derivatized with vanillin-sulphuric acid reagent showed 8 spots.

HPTLC finger print profile of ethanol extract of the roots of *H. vitifolius*

HPTLC finger print profile of ethanol extract showed 8 peaks (Fig. 8) of which 4 were major peaks at *Rf* 0.30, 0.68, 0.88 and 0.92 whereas peaks at Rf 0.12, 0.41, 0.54 and 0.63 are moderately smaller peaks.



UV- 254 nm UV – 366 nm Visible light (After derivatization with V-S reagent)

Fig. 7: TLC of ethanol extract of the roots of *H. vitifolius*

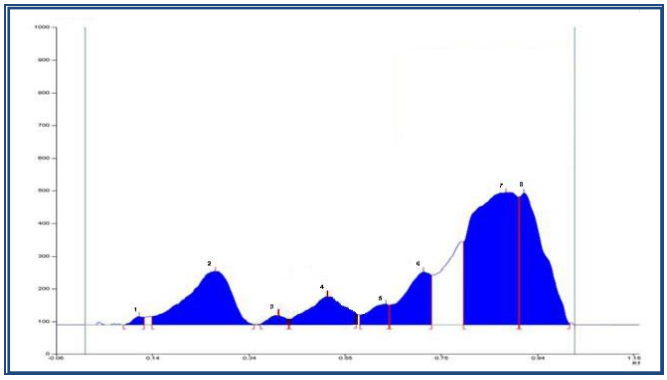


Fig. 8: HPTLC finger print profile of the ethanol extract of the roots of *H. vitifolius*

DISCUSSION

The various analytical standards evaluated provide useful data in standardization and quality control of the drug. The results of preliminary phytochemical screening of *H. vitifolius* root revealed the presence of alkaloids, flavonoids and saponins in chloroform, ethyl acetate and alcohol extracts whereas steroids was found in n-hexane, chloroform, ethyl acetate and alcohol extracts. Tannin and glycosides were found in ethyl acetate and alcohol extracts whereas triterpenoids found were found in only chloroform and ethyl acetate extracts.

Herbal medicines are composed of many constituents and are therefore capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the herbal medicine.

The obtained TLC/HPTLC fingerprinting profile of various extracts of *H. vitifolius* will be used as a quality control tool and will play a major role in herbal drug standardization in proper identification of the plant.

CONCLUSION

The data of physicochemical parameters, preliminary phytochemical analysis, TLC/HPTLC finger print analysis of the roots of *H. vitifolius* will be useful in the standardization and validation of the drug.

Conflict of interest statement

We declare that we have no conflict of interest.

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