

HPTLC Fingerprinting of *Pandanus tectorius* Leaves as a Quality Control Parameter

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Abstract: *Pandanus tectorius* leaves are used in skin disease, small pox, scabies and leprosy. They also possess antimicrobial, antifungal, antiviral, anthelmintic, anti-epileptic, anti-inflammatory, antioxidant, anticancer, analgesic and CNS-depressant activity. Such uses make it a potential candidate in herbal extracts and formulations. However, no chromatographic fingerprint for identification or evaluation of its leaf is available. The aim of the present work is to develop an HPTLC fingerprint of *P. tectorius* leaves which can be used as a quality control parameter. Leaves of *P. tectorius* were collected, authenticated, defatted and then extracted with methanol. After mobile phase development, HPTLC was performed in which R_f and Area Under Curve were calculated. HPTLC fingerprinting of methanol extract showed sixteen distinct peaks at UV 254nm and eighteen distinct peaks at UV 366nm using the mobile phase Toluene: Methanol: Formic acid (8:2:1). This work could be helpful to herbal industry as an important standardization parameter, in detection of adulteration and serve as a guide for isolation of phytoconstituents from *P. tectorius* leaves.

Keywords: Fingerprinting, HPTLC, *Pandanus fascicularis*, *Pandanus odoratissimus*, *Pandanus tectorius*.

Introduction

Pandanus tectorius syn *P. fascicularis* and *P. odoratissimus* (Pandanaceae) is traditionally known as Ketaki and Kewda (Fig. 1). It is mainly found along sea coasts of India. Its leaves are used in skin disease, small pox, scabies and leprosy¹. They also possess antimicrobial, antifungal, antiviral, anthelmintic, anti-epileptic, anti-inflammatory, antioxidant, anticancer, analgesic and CNS-depressant activity, the investigations of which were mainly done using the methanolic extract². The leaves specifically contain piperidine alkaloids¹. Their hydroalcoholic extract also contains glycosides, triterpenoids, saponins, flavonoids and phenolics³. Thus, *P. tectorius* leaf is a potential candidate for herbal extracts and formulations. However, no chromatographic fingerprint for identification, evaluation and standardization of its leaf is available. Considering that the methanolic extract of *P. tectorius* leaf was mainly used in proving the pharmacological actions and the leaves contain several important classes of secondary metabolites, the present work focuses on developing an HPTLC fingerprint of methanolic extract of *P. tectorius* leaf.



Figure 1. *Pandanus tectorius*

Experimental

Collection and authentication

Collection of leaves was done in August 2014 from School of Pharmacy, RK University. Herbarium was prepared and authenticated by Botanist of School of Science, RK University.

Extraction

Leaves were dried in oven at 60°C. 500g dried leaf powder was first defatted with petroleum ether and then extracted with 500ml methanol for 2h at 60°C in round bottom flask. Extract was filtered and evaporated to dryness on water bath at 60°C.

Development of mobile phase

Pilot TLC were developed for Methanol extract using various mobile phases like Toluene: Methanol (9:1 to 1:9) and Chloroform: Methanol (9:1 to 1:9) followed by addition of ammonia, ethyl acetate, formic acid and acetic acid. After observing the pilot results, mobile phases Toluene : Methanol : Ammonia (8:2:1 to 6:4:1), Toluene : Methanol : Formic acid (8:2:1 to 6:4:1), Chloroform : Methanol : Ammonia (8:2:1 to 4:6:1), Chloroform : Methanol : Formic Acid (7:3:1 to 4:6:1), Chloroform : Water : Ammonia (2:8:1 to 6:4:1), Chloroform : Water : Formic Acid (6:4:1 to 3:7:1), Toluene : Methanol : Ethyl Acetate (8:2:1 to 6:4:1), Chloroform : Methanol : Ethyl Acetate (8:2:1 to 0.5:9.5:1) were selected for developing a proper fingerprint. All plates were observed under visible light, UV 254nm and UV 366nm.

HPTLC fingerprinting

Toluene: Methanol: Formic acid (8:2:1) gave the most suitable TLC fingerprint. HPTLC fingerprinting of methanol extract using this mobile phase was done at National Facility for Drug Discovery, Dept. of Chemistry, Saurashtra University, Rajkot under the following conditions...

Stationary phase: Silica gel 60 F 254 (E. Merck KGaA)

Sample application: CAMAG Linomat 5

Detection: CAMAG TLC Scanner 3

Lamp: D2 & W

Measurement type: Remission

Measurement mode: Absorption

Optical filter: Second order

Data filtering: Savitsky-Golay 7

Two tracks of same extract at different concentrations were run for the HPTLC fingerprinting and scanned under visible light, UV 254nm and UV 366nm.

Results and Discussion

Sixteen peaks were detected at 254nm (R_f 0.04, 0.12, 0.13, 0.20, 0.22, 0.24, 0.31, 0.34, 0.35, 0.41, 0.50, 0.57, 0.65, 0.71, 0.74, 0.78; Table 1, Fig. 2-4) and eighteen peaks were detected at 366nm (R_f 0.01, 0.02, 0.03, 0.12, 0.19, 0.22, 0.27, 0.29, 0.32, 0.35, 0.37, 0.39, 0.41, 0.45, 0.52, 0.59, 0.66, 0.69; Table 2, Fig. 5-7) upon HPTLC of methanol extract of *P. tectorius* leaves using mobile phase Toluene: Methanol: Formic acid (8:2:1).

Table 1. R_f & Area Under Curve of HPTLC of Methanol extract at 254nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	6.1 AU	0.04 Rf	614.2 AU	9.22 %	0.11 Rf	94.7 AU	45411.1 AU	2.67 %
2	0.11 Rf	95.0 AU	0.12 Rf	107.9 AU	6.89 %	0.13 Rf	84.2 AU	1174.0 AU	1.88 %
3	0.13 Rf	84.6 AU	0.13 Rf	115.2 AU	7.36 %	0.15 Rf	73.9 AU	1699.1 AU	2.72 %
4	0.18 Rf	64.9 AU	0.20 Rf	80.1 AU	5.12 %	0.21 Rf	74.1 AU	1654.0 AU	2.65 %
5	0.21 Rf	74.2 AU	0.22 Rf	76.4 AU	4.88 %	0.23 Rf	60.4 AU	1193.5 AU	1.91 %
6	0.23 Rf	61.0 AU	0.24 Rf	64.9 AU	4.14 %	0.26 Rf	49.4 AU	1153.9 AU	1.85 %

7	0.30 Rf	42.7 AU	0.31 Rf	80.7 AU	5.15 %	0.32 Rf	55.2 AU	793.6 AU	1.27 %
8	0.32 Rf	56.2 AU	0.34 Rf	84.1 AU	5.37 %	0.34 Rf	68.9 AU	1422.8 AU	2.28 %
9	0.34 Rf	67.5 AU	0.35 Rf	74.3 AU	4.75 %	0.37 Rf	33.4 AU	1101.4 AU	1.76 %
10	0.40 Rf	7.8 AU	0.41 Rf	25.1 AU	1.6 %	0.44 Rf	13.1 AU	484.3 AU	0.78 %
11	0.47 Rf	3.9 AU	0.50 Rf	23.7 AU	1.52 %	0.50 Rf	5.6 AU	294.7 AU	0.47 %
12	0.54 Rf	10.3 AU	0.57 Rf	39.9 AU	2.55 %	0.58 Rf	17.4 AU	546.9 AU	0.88 %
13	0.59 Rf	21.9 AU	0.65 Rf	55.8 AU	3.57%	0.66 Rf	42.0 AU	1968.5 AU	3.15 %
14	0.68 Rf	50.3 AU	0.71 Rf	66.7 AU	4.26 %	0.74 Rf	33.5 AU	2750.1 AU	4.40 %
15	0.74 Rf	34.0 AU	0.74 Rf	35.2 AU	2.25 %	0.77 Rf	10.9 AU	715.4 AU	1.14 %
16	0.78 Rf	9.6 AU	0.78 Rf	21.6 AU	1.38 %	0.79 Rf	0.2 AU	122.5 AU	0.20 %

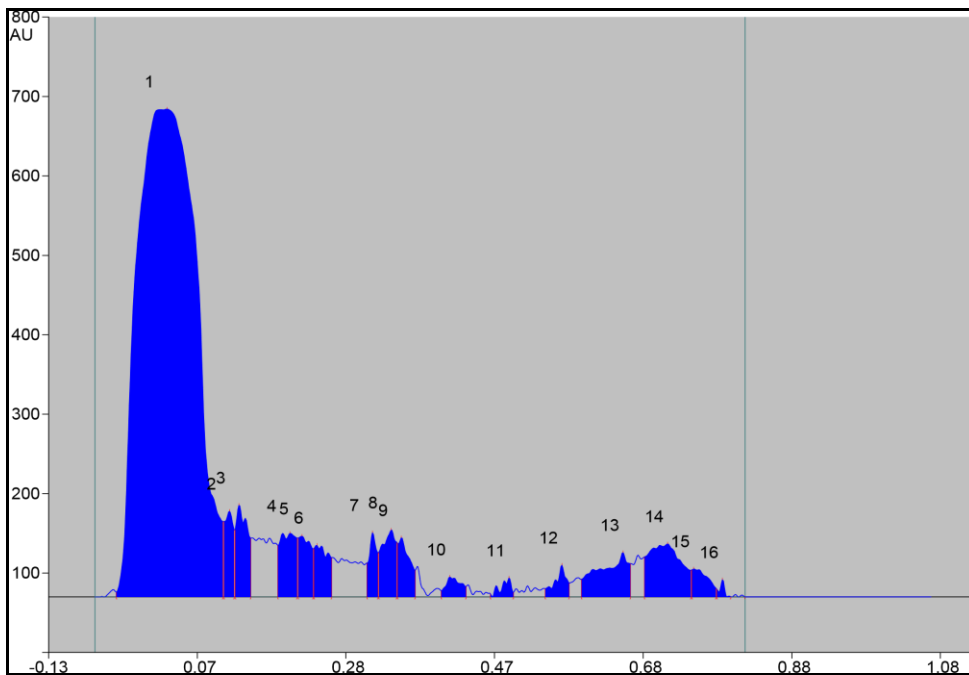


Figure 2. HPTLC chromatogram of Methanol extract at 254nm

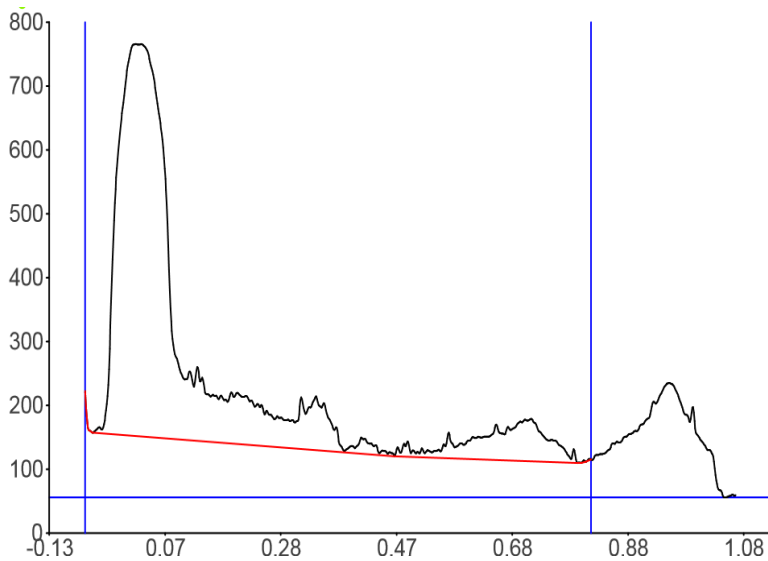


Figure 3. HPTLC of Methanol extract at 254nm

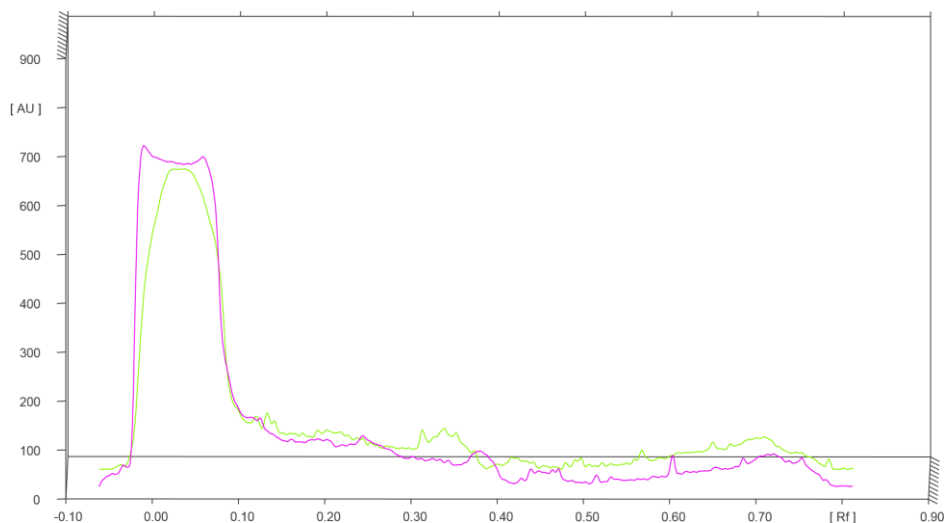


Figure 4. HPTLC densitometric superimposable chromatogram of Methanol extract at 254nm

Table 2. R_f & Area Under Curve of HPTLC of Methanol extract at 366nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.8 AU	0.01 Rf	28.7 AU	5.16 %	0.02 Rf	7.2 AU	152.4 AU	1.57 %
2	0.02 Rf	12.1 AU	0.02 Rf	24.1 AU	4.34 %	0.03 Rf	4.1 AU	347.4 AU	3.57%
3	0.03 Rf	5.0 AU	0.03 Rf	237.4 AU	2.69 %	0.06 Rf	25.1AU	6964.3 AU	1.54 %
4	0.11 Rf	2.6 AU	0.12 Rf	16.6 AU	2.99 %	0.12 Rf	0.8 AU	96.0 AU	0.99 %
5	0.18 Rf	2.8 AU	0.19 Rf	22.7 AU	4.08 %	0.21 Rf	8.5 AU	314.6 AU	3.23 %
6	0.21 Rf	9.4 AU	0.22 Rf	17.8 AU	3.20 %	0.24 Rf	6.9 AU	226.5 AU	2.33 %
7	0.25 Rf	0.6 AU	0.27 Rf	13.4 AU	2.42 %	0.27 Rf	0.9 AU	94.8 AU	0.97 %
8	0.27 Rf	1.3 AU	0.29 Rf	15.8 AU	2.84 %	0.30 Rf	10.1AU	185.8 AU	1.91 %
9	0.31 Rf	3.3 AU	0.32 Rf	18.7 AU	3.37 %	0.33 Rf	1.3 AU	152.7 AU	1.57 %
10	0.34 Rf	1.5 AU	0.35 Rf	14.4 AU	2.59 %	0.35 Rf	8.5 AU	94.6 AU	0.97 %
11	0.36 Rf	1.0 AU	0.37 Rf	26.2 AU	4.72 %	0.38 Rf	9.1 AU	157.6 AU	1.62 %
12	0.38 Rf	9.8 AU	0.39 Rf	22.9 AU	4.12 %	0.40 Rf	6.3 AU	238.2 AU	2.45 %
13	0.40 Rf	7.7 AU	0.41 Rf	26.7 AU	4.81 %	0.43 Rf	6.5 AU	297.9 AU	3.06 %
14	0.44 Rf	2.2 AU	0.45 Rf	13.5 AU	2.42 %	0.46 Rf	0.7 AU	67.4 AU	0.69 %
15	0.51 Rf	0.5 AU	0.52 Rf	15.4 AU	2.77 %	0.53 Rf	2.1 AU	88.9 AU	0.91 %
16	0.59 Rf	0.6 AU	0.59 Rf	11.3 AU	2.03 %	0.60 Rf	1.7 AU	55.2 AU	0.57 %
17	0.66 Rf	1.9 AU	0.66 Rf	13.8 AU	2.48 %	0.67 Rf	3.6 AU	101.1 AU	1.04 %
18	0.68 Rf	2.9 AU	0.69 Rf	16.5 AU	2.97 %	0.70 Rf	0.6 AU	99.1 AU	1.02 %

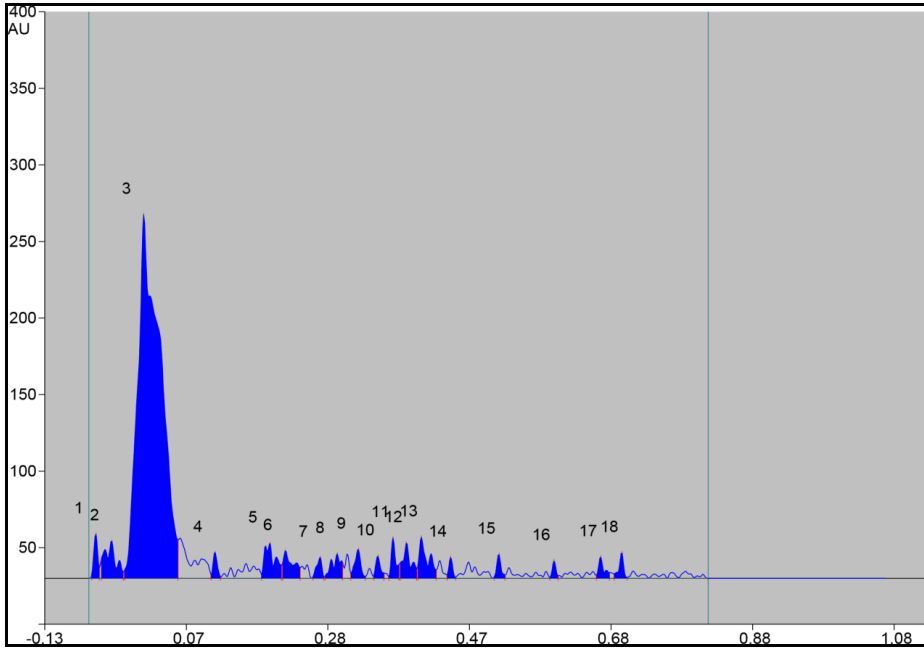


Figure 5. HPTLC chromatogram of Methanol extract at 366nm

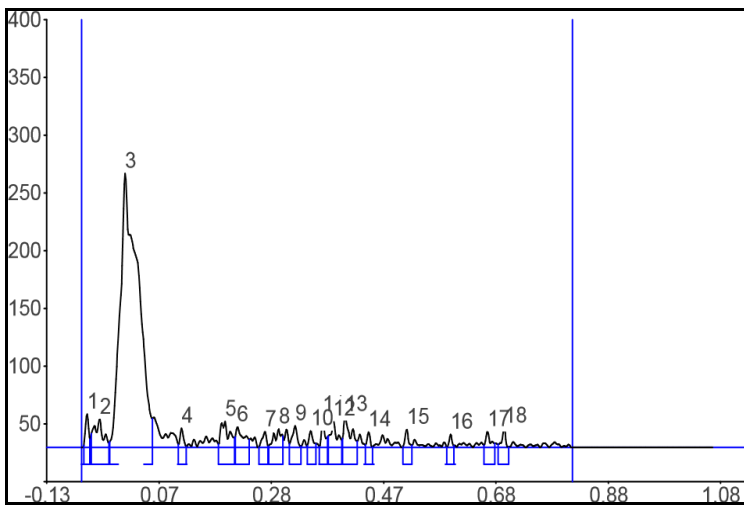


Figure 6. HPTLC of Methanol extract at 366nm

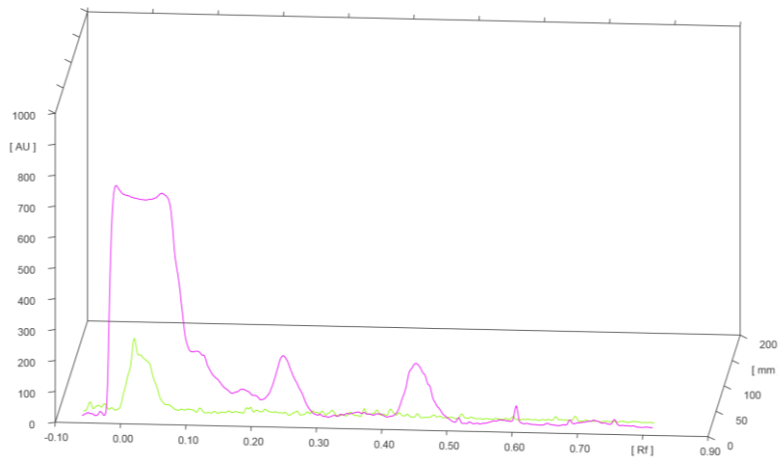


Figure 7. HPTLC densitometric superimposable chromatogram of Methanol extract at 366nm

The present work can be helpful to herbal industry as an important standardization parameter of *P. tectorius*. It can be very useful for authentication of raw material and in detection of adulteration of its dried leaves as well as its methanolic extract. The work can also guide the isolation of phytocompounds from *P. tectorius* leaves.

References

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