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# HPTLC Fingerprinting of *Scaevola frutescens* Leaves as a Quality Control Parameter

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**Abstract:** *Scaeovola frutescens* leaves are digestive, carminative and applied externally on tumors. They also possess antipyretic, anti-inflammatory, anticoagulant, antibacterial, antifungal, diuretic, skeletal muscle relaxant and antioxidant activity. Such uses make it potentially useful in herbal formulations. However, no chromatographic fingerprint for its identification or evaluation is available. The aim of this work is to develop an HPTLC fingerprint of *S. frutescens* leaves as a quality control parameter. Leaves of *S. frutescens* were defatted and subjected to chloroform extraction. After mobile phase development involving several pilot TLC, the result showing distinct spots was further subjected to HPTLC analysis. $R_f$ and Area Under Curve were calculated. HPTLC fingerprinting of Chloroform extract showed nine distinct peaks each at UV 254nm and UV 366nm using the mobile phase Chloroform: Methanol: Ethyl acetate (9:1:1). 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 *S. frutescens* leaves.
**Keywords:** Fingerprinting, HPTLC, Scaevola frutescens, Scaevola koenigii, Scaevola taccada.

## Introduction

*Scaevola frutescens* syn. *S. koenigii* and *S. taccada* (Goodeniaceae) is traditionally known as Bhadraaksha (Fig. 1). It is mainly found along sea coasts of India. Its leaves are digestive, carminative and applied externally on tumors and swollen legs. Leaf decoction is used in tachycardia[1]. The leaves also possess antipyretic, anti-inflammatory, anticoagulant, antibacterial, antifungal, diuretic, skeletal muscle relaxant and antioxidant activity[2, 3]. Its aerial parts contain loganin, sylvestroside-III and cantleyoside[1]. Thus, *S. frutescens* leaf is a potential candidate in herbal formulations. However, no chromatographic fingerprint for its identification, evaluation and standardization is available. Currently the herbal market is growing exponentially and Govt. of India has set strict guidelines for quality control in herbal industries. The present work focuses on developing an HPTLC fingerprint of *S. frutescens* leaf.

**Figure 1.** ***Scaevola frutescens***

## Experimental

### Collection and authentication

Collection of leaves was done in July 2014 from Medicinal Garden of School of Pharmacy, RK University. Herbarium was authenticated by Botanist, School of Science, RK University.

### Extraction

Leaves were dried in oven at 60°C and powdered in grinder. 500g dry leaf powder was first defatted with petroleum ether and then extracted with 500ml Chloroform for 3 hours 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 Chloroform 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 for removal of tailing. After observing the pilot results, mobile phases Toluene : Methanol : Formic acid (9.5:0.5:1 to 8.5:1.5:1), Toluene : Methanol : Ammonia (9.5:0.5:1 to 8.5:1.5:1), Chloroform : Methanol : Ethyl Acetate (9.5:0.5:1 to 8.5:1.5:1), Toluene : Methanol : Acetic acid (9.5:0.5:1 to 8.5:1.5:1) and Chloroform : Methanol : Formic acid (7:1:1 to 13:1:1) were selected for developing a proper fingerprint. All plates were observed under visible light, UV 254nm and UV 366nm.

### HPTLC fingerprinting

Chloroform: Methanol: Ethyl Acetate (9:1:1) gave the most suitable TLC fingerprint. HPTLC fingerprinting of chloroform 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

Four 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

Nine peaks were detected at 254nm ($R_f$ 0.02, 0.12, 0.22, 0.25, 0.30, 0.41, 0.50, 0.73, 0.78; Table 1, Fig. 2-4) and nine peaks were detected at 366nm ($R_f$ 0.01, 0.02, 0.07, 0.12, 0.23, 0.30, 0.42, 0.50, 0.80; Table 2, Fig. 5-7) upon HPTLC of chloroform extract of *S. frutescens* leaves using mobile phase Chloroform: Methanol: Ethyl Acetate (9:1:1).

**Table 1. $R_f$ & Area Under Curve of HPTLC of Chloroform extract at 254nm**

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.01 Rf | 0.3 AU | 0.02 Rf | 385.3 AU | 38.89 % | 0.09 Rf | 8.0 AU | 6655.7 AU | 45.44 % |
| 2 | 0.10 Rf | 68.2 AU | 0.12 Rf | 93.2 AU | 9.41 % | 0.18 Rf | 6.3 AU | 3462.6 AU | 9.45 % |
| 3 | 0.18 Rf | 16.3 AU | 0.22 Rf | 45.8 AU | 4.63 % | 0.24 Rf | 9.7 AU | 1439.7 AU | 3.93 % |
| 4 | 0.24 Rf | 30.6 AU | 0.25 Rf | 33.2 AU | 3.35 % | 0.27 Rf | 0.6 AU | 436.4 AU | 1.19 % |
| 5 | 0.29 Rf | 11.8 AU | 0.30 Rf | 13.4 AU | 1.35 % | 0.33 Rf | 7.2 AU | 294.7 AU | 0.80 % |
| 6 | 0.37 Rf | 18.6 AU | 0.41 Rf | 122.4 AU | 12.36 % | 0.46 Rf | 8.3 AU | 4270.8 AU | 11.65 % |
| 7 | 0.46 Rf | 10.0 AU | 0.50 Rf | 29.0 AU | 2.93 % | 0.53 Rf | 4.5 AU | 989.0 AU | 2.70 % |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 8 | 0.71 Rf | 2.1 AU | 0.73 Rf | 10.8 AU | 1.09 % | 0.75 Rf | 0.3 AU | 208.1 AU | 0.57 % |
| 9 | 0.76 Rf | 0.5 AU | 0.78 Rf | 257.6 AU | 26.00 % | 0.83 Rf | 6.9 AU | 8896.4 AU | 24.27 % |

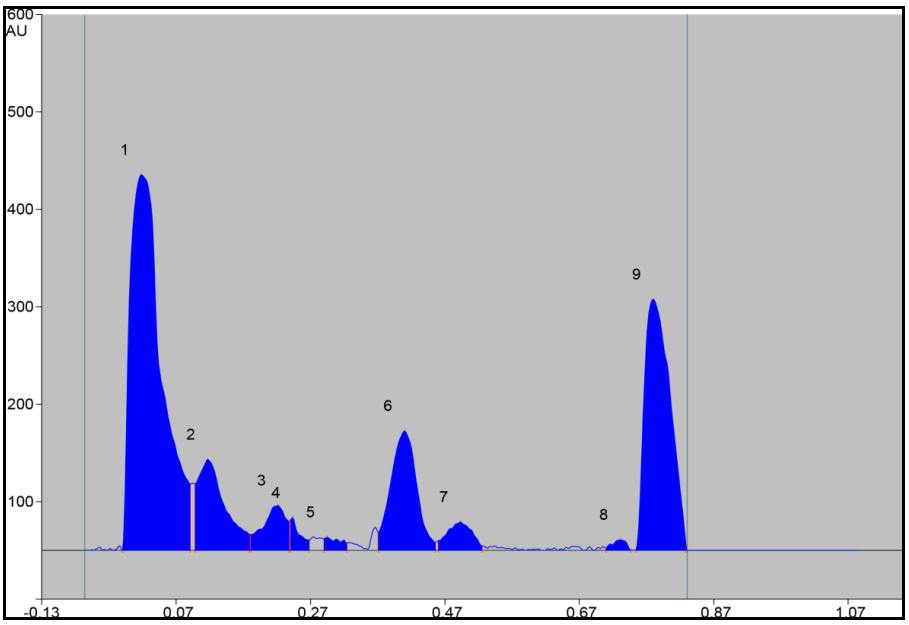


**Figure 2. HPTLC chromatogram of Chloroform extract at 254nm**

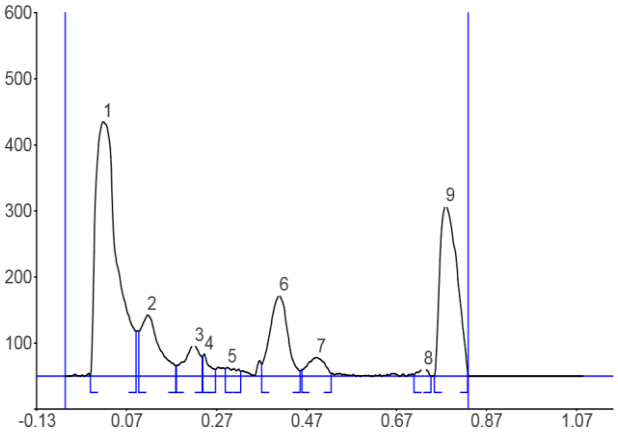


**Figure 3. HPTLC of Chloroform extract at 254nm**

**Table 2. $R_f$ & Area Under Curve of HPTLC of Chloroform extract at 366nm**

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 Rf | 0.6 AU | 0.01 Rf | 17.7 AU | 0.98 % | 0.02 Rf | 0.8 AU | 100.1 AU | 0.15 % |
| 2 | 0.01 Rf | 4.3 AU | 0.02 Rf | 440.2 AU | 24.48% | 0.06 Rf | 216.8AU | 16657.3AU | 24.31% |
| 3 | 0.06 Rf | 219.3AU | 0.07 Rf | 232.0 AU | 12.90% | 0.09 Rf | 156.6AU | 5421.9 AU | 7.91 % |
| 4 | 0.09 Rf | 156.8AU | 0.12 Rf | 317.6 AU | 17.66% | 0.17 Rf | 73.5 AU | 12064.6AU | 17.61 % |
| 5 | 0.19 Rf | 62.4 AU | 0.23 Rf | 182.6 AU | 10.16% | 0.28 Rf | 62.8 AU | 7856.4 AU | 11.46 % |
| 6 | 0.29 Rf | 60.2 AU | 0.30 Rf | 69.4 AU | 3.86 % | 0.32 Rf | 54.7 AU | 1370.8 AU | 2.00 % |
| 7 | 0.35 Rf | 49.9 AU | 0.42 Rf | 350.6 AU | 19.50% | 0.47 Rf | 69.4 AU | 16796.7AU | 24.51 % |
| 8 | 0.47 Rf | 69.7 AU | 0.50 Rf | 117.5 AU | 6.53 % | 0.56 Rf | 9.0 AU | 5077.5 AU | 7.41 % |
| 9 | 0.72 Rf | 0.9 AU | 0.80 Rf | 70.8 AU | 3.94 % | 0.83 Rf | 2.3 AU | 3180.0 AU | 4.64 % |

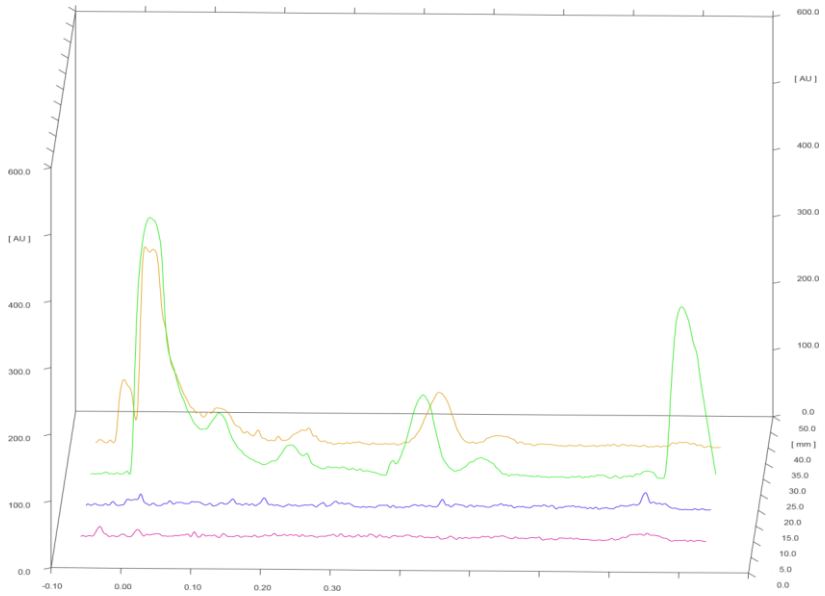

**Figure 4. HPTLC densitometric superimposable chromatogram of Chloroform extract at 254nm**

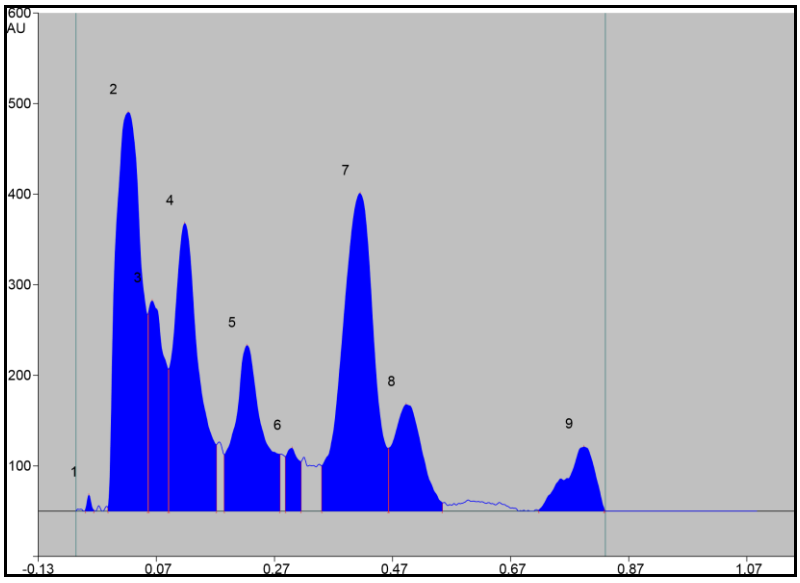


**Figure 5. HPTLC chromatogram of Chloroform extract at 366nm**

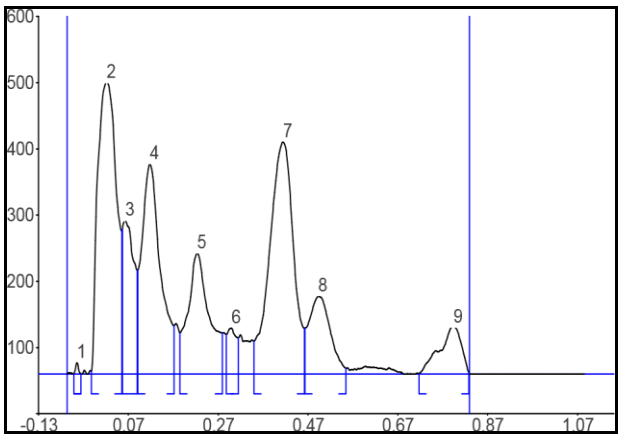


**Figure 6. HPTLC of Chloroform extract at 366nm**

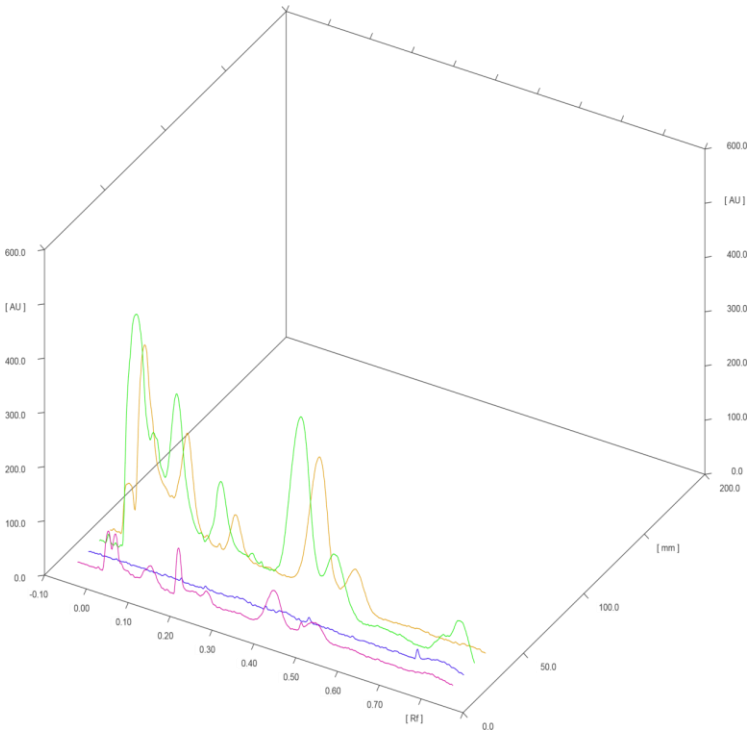


**Figure 7. HPTLC densitometric superimposable chromatogram of Chloroform extract at 366nm**

The present work contains sufficient chromatographic data which can be helpful to herbal industry as an important standardization parameter of *S. frutescens* leaves. It can be specifically useful for authentication of raw material of the leaves and in detection of adulteration. The work, if followed up by column chromatography or preparative TLC, can also serves as a guide for isolation of phytoconstituents from *S. frutescens* leaves.

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