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HIGH PRESSURE THIN LAYER CHROMATOGRAPHY FINGERPRINTING ANALYSIS OF AN AYURVEDIC HERB KASAMARDA MOOLA - *CASSIA OCCIDENTALIS* (ROOT)

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ABSTRACT

India has an ancient history of the use of plants in the indigenous system of medicine dating back to over 5000 years. It has been estimated that over 8000 plants are used in traditional, folk and herbal medicine, *Cassia occidentalis* Linn. (Caesalpiniaceae), a perennial plant of southern India, is an Ayurvedic plant which is used in several traditional medicines to cure various diseases. The parts of the plant used are roots, leaves and seeds. Leaves of Cassia occidentalis plant have ethno medicinal importance like paste of leaves is externally applied on healing wounds, sores, itch, cutaneous diseases, bone fracture, ringworm, skin diseases and throat infection. The plant is used for fever, menstrual problems, tuberculosis, diuretic anaemic, liver complaints, and as a tonic for general weakness and illness and is also reported to cure leprosy. An infusion of the plant bark is given by the folklore in diabetes. WHO guidelines emphasise the need for modern and sophisticated modern instrumental method like HPTLC to standardize herbal drugs. HPTLC study was carried out ethanol extract detected at 254 nm. The ethanolic extract of root of *Cassia occidentalis* Linn. are subjected to HPTLC and developed the fingerprint pattern. HPTLC fingerprinting of Kasamarda moola(*Cassia occidentalis* root) provides useful information regarding quality control parameters and identifying parameters to substantiate and authenticate the drug and could be used for comparison of market samples to ensure its genuineness It can be concluded that HPTLC fingerprint analysis of Cassia occidentalis Linn. can be used for Standardisation.

KEYWORDS: Kasamarda, Cassia occidentalis, HPTLC fingerprinting, Standardization.

INTRODUCTION

Kasamarda is an herb which grows up to 2 m and possesses yellow flowers. It is found all over India on road sides as weed. Though Charaka omitted it among the ganas, Sushruta and Vagbhata have included it under Sursadigana. Vagbhata denoted it with a synonym kasaghna. The drug Kasamarda is used in the indigenous system of medicine since long time. Charak mentioned its use for curing cough. It has been mentioned in various nighantus viz. Rajnighantu, Dhanwantari, Bhavaprakasha, Rajballabha and others as bitter, sweet light, hot, Appetiser, aphrodisiac, stomachic and diuretic.[1]

MATERIAL AND METHODS

HPTLC instrument (make CAMAG), Merck, TLC Silica gel 60 F 254, Toluene (HPLC grade), Methanol (HPLC grade). The method was validated using the following parameters; specificity, precision, repeatability, intermediate precision, and robustness.[2]

METHODOLOGY

Instrumental and experimental conditions

Sample preparation: 5 g of drug sample was taken in 100 ml of ethanol for extract preparation. Apply the ethanol extract (4 µl each) on TLC plate. TLC plate was developed using Toluene: Ethyl acetate (8: 2, *v/v*) as mobile phase. After development allows the plate to dry in air and examine under UV (366 nm). Dip the plate in vanillin-sulphuric acid reagent followed by heating at 110°C about 5 min and observe under visible light, the plate shows various major spots and photographed as depicted in Fig. 1.

HPTLC instrumental conditions

Scanner type	Multiple λ
Optimization for	Resolution
Measurement mode	Advanced
Quick scan start	6.5 mm
Quick scan end	86.5 mm
Sensitivity	Automatic
0 adjust position Y	6.5 mm
0 adjust track	Track 1
Scanning speed	20 mm/s
Data resolution	100 µm/step
Slit	5 x 0.2 mm, micro
Lamp	Deuterium & Tungsten
Wavelength(s)	254 nm (Absorbance, Lamp: Deuterium & Tungsten, Filter: K400) 366 nm (Fluorescence, Lamp: Mercury, Filter: K400)
Spray gas	N_2
Filling speed	15µL/s
Predosage volume	200nL
Retraction volume	200nL
Dosage speed	110nL/s
Rinsing cycles/vacuum	2/6 s
Filling cycles/vacuum	2/0s
Stationery Phase	Merck, HPTLC Silica gel 60 F_{254}
Plate format	100 x 100 mm
Application	Position Y: 10.0 mm, length: 10.0mm, width: 0mm
Track	First Position X: 30.0 m, distance 20.0 mm
Solvent front position	80 mm
Development Chamber parameters	
Tank	TTC 10 x 10
Mobile phase	**Toluene: Ethyl acetate** (8:2 v/v)
Saturation time	20 min
Volume front through	5 mL
Volume rear through	5 mL
Drying time	5 min
Drying temperature	Room temperature

TLC plate after derivatization with Vanillin sulphuric acid reagent

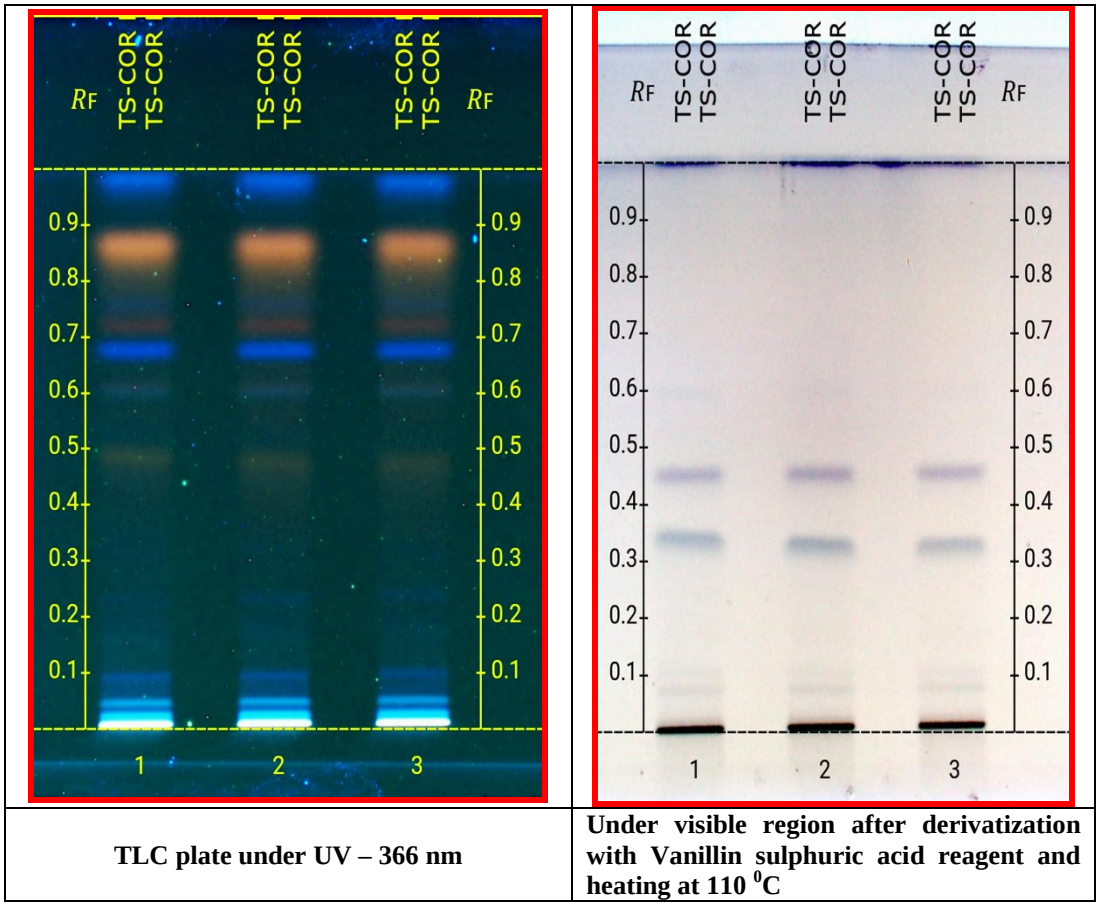
Derivatization Reagent	**Vanillin Sulphuric acid reagent**
Dipping speed	5
Dipping time	1 s
Heating	110 ^{0}C for 1-3 min, heated after

Scan Conditions

Scanner type	Multiple λ
Optimization for	Resolution
Measurement mode	Advanced
Detector mode	Manual
Quick scan start	6.5 mm
Quick scan end	86.5 mm
Quick scan track	All tracks
Analog offset	10%

Sensitivity	Automatic
0 adjust position Y	6.5 mm
Scanning speed	20 mm/s
Data resolution	100 µm/step
Slit	5 x 0.2 mm, micro
Lamp	Deuterium & Tungsten
Wavelength(s)	520 nm (Absorbance, Lamp: Deuterium & Tungsten, Filter: K320)

RESULTS



TLC plate under UV – 366 nm | **Under visible region after derivatization with Vanillin sulphuric acid reagent and heating at 110 ^{0}C**

Fig. 1: TLC plate photograph of COR ethanol extract.

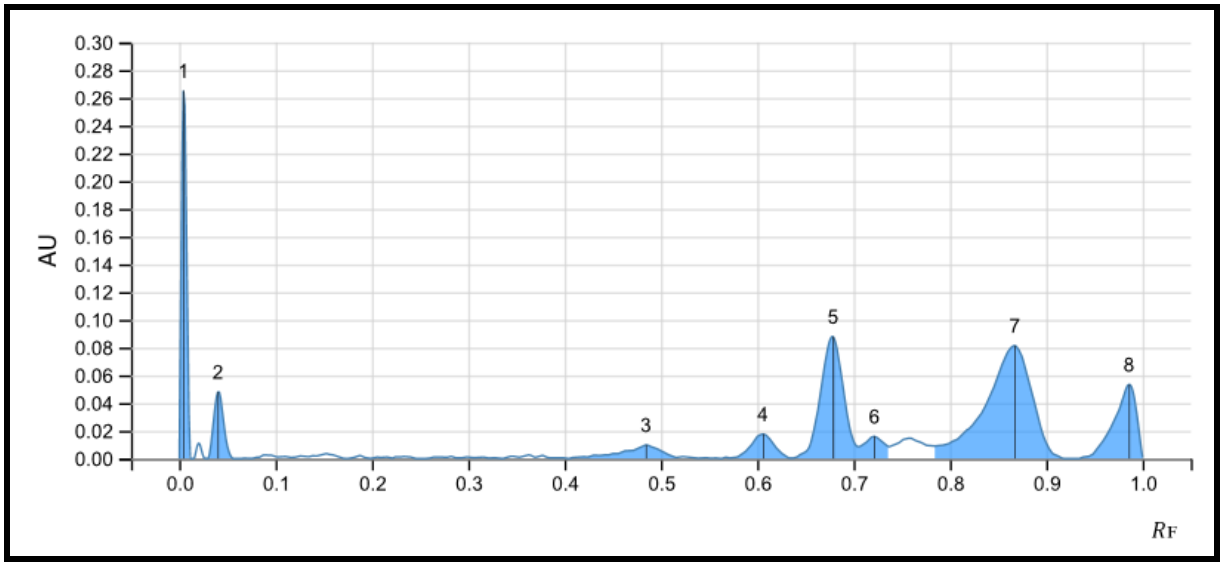


Fig. 2: HPTLC chromatogram of COR ethanol extract scanned at 366 nm.

HPTLC chromatogram is obtained for the COR ethanol extract upon scanned at 366 nm wavelength as shown in fig. 2. The corresponding peak list and Rf values for the chromatogram is depicted in table 1. The COR ethanol extract showed eight spots upon detection at UV 366 nm wavelength at Rf values as 0.004, 0.040, 0.484, 0.606, 0.679, 0.721, 0.867, 0.986.

Table 1: Peak list and Rf values of COR ethanol extract obtained at 366 nm.

Peaks	Max *R*□	Max pos. (mm)	Height	Height %	Area	Area %
1	0.004	10.3	0.2652	45.75	0.00168	14.02
2	0.040	12.8	0.0480	8.29	0.00057	4.73
3	0.484	43.9	0.0099	1.71	0.00046	3.81
4	0.606	52.4	0.0177	3.05	0.00050	4.16
5	0.679	57.5	0.0879	15.17	0.00243	20.23
6	0.721	60.5	0.0160	2.77	0.00039	3.22
7	0.867	70.7	0.0814	14.05	0.00455	37.92
8	0.986	79.0	0.0534	9.22	0.00143	11.90

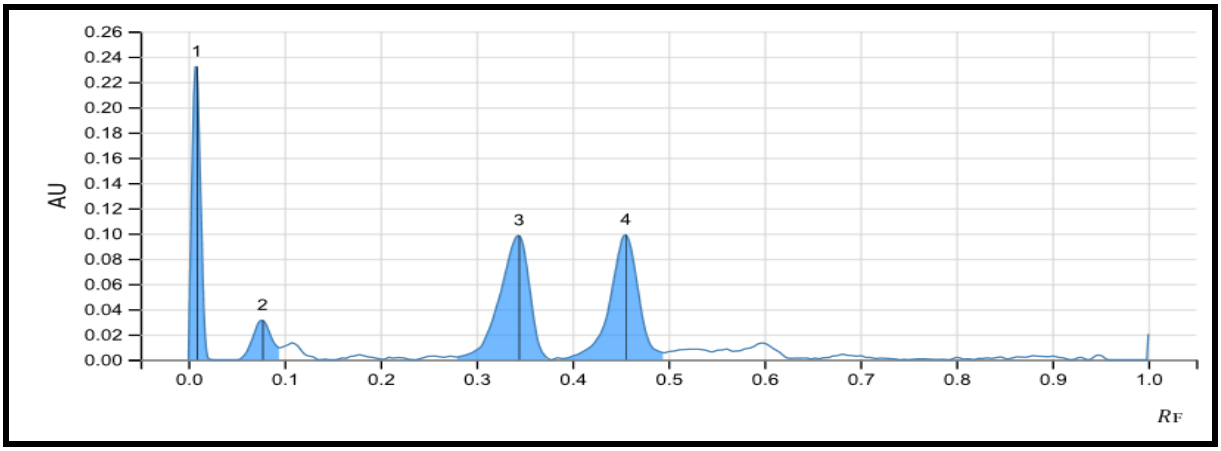


Fig. 3 HPTLC chromatogram of COR ethanol extract scanned at 520 nm after derivatizing with vanillin sulphuric acid reagent.

HPTLC chromatogram is obtained for the COR ethanol extract upon scanned at 520 nm wavelength after derivatizing with vanillin sulphuric acid reagent as shown in fig. 3. The corresponding peak list and Rf values for the chromatogram as depicted in table 2. The COR ethanol extract showed four spots upon scanning the TLC plate at 520 nm wavelength showing Rf values as 0.009, 0.077, 0.344, 0.456.

Table 2: Peak list and Rf values of COR ethanol extract obtained at 520 nm after derivatizing with vanillin sulphuric acid reagent.

Peaks	Max *R*□	Max pos. (mm)	Height	Height %	Area	Area %
1	0.009	10.6	0.2321	50.39	0.00242	24.02
2	0.077	15.4	0.0313	6.79	0.00071	7.08
3	0.344	34.1	0.0984	21.36	0.00354	35.09
4	0.456	41.9	0.0989	21.47	0.00341	33.81

CONCLUSION

The *Cassia occidentalis* (Root) was subjected to HPTLC fingerprint analysis in ethanol extract showing various phytoconstituents under different detection system. HPTLC fingerprinting of Kasamarda moola (*Cassia occidentalis* root) provides useful information regarding quality control parameters and identifying parameters to substantiate and authenticate the drug and could be used for comparison of market samples to ensure its genuineness. A simple, specific and accurate HPTLC method was validated for its fingerprint analysis. The developed method can be used for the quality control purpose for the authentication of the root of the plant species.

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