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HPTLC Fingerprinting of *Medicago sativa* root extract as a Quality Control Parameter

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Abstract : *Medicago sativa* Linn. is known as the “father of all foods” (*al-fal-fa*). *Medicago sativa* used in Ayurvedic, Homoeopathic and Chinese system of medicine in central nervous, digestive system disorders, and for the treatment of various other ailments. The present work focuses on developing a simple HPTLC fingerprint of *Medicago sativa* root extract. Successive maceration was done in increasing order of polarity and toluene, chloroform, methanol and water extracts were prepared. Methanol root extract was used to develop a suitable mobile phase for fingerprinting. Mobile phase development involved several pilot TLC. The mobile phase showing distinct spots in TLC was found to be Chloroform: Methanol: Ethyl acetate (1:5:5). It was further subjected to HPTLC fingerprinting where R_f and Area Under Curve were calculated. HPTLC fingerprinting showed 8 peaks at 254nm and 6 peaks at 366nm. This work provides a simple technique for standardization and detection of adulteration of *Medicago sativa* root extract and preparations, consumed by people for treatment of various disease conditions.

Keywords : Alfalfa, *Medicago sativa*, HPTLC, Quality control, Lucerne.

Introduction

Medicago sativa Linn. known as the “father of all foods” (*al-fal-fa*), is a perennial herbaceous leguminous plant species that originated in Asia.¹ According to Ayurveda, homeopathy and modern research, it possess antioxidant, free radical scavenging, antibacterial, and hypocholesteromic and anti-wrinkle activity.²⁻⁶ According to Chinese system of traditional medicine it is used to reduce fever, improve urine flow, and treat jaundice, kidney stones, and night blindness.⁷ Xiong demonstrated that Extracts prepared from *M. sativa* roots may be used to prepare medical preparations like powder, pill, or decoction for lowering the levels of cholesterol and lipid in blood, improving the liver function and the control and transmission of nerve tissue, and treating calculus.⁸ The main objective of this study was to evaluate and optimize the HPTLC fingerprint method in standardization of *Medicago sativa* to provide beneficial information in regarding the standardization according to WHO guidelines. The present work focuses on developing an HPTLC fingerprint of *M. sativa* roots.



Figure 1. *Medicago sativa* roots

Experimental⁹⁻²²

Crude drug material collection

Medicago sativa whole plant was (12 to 18 inch ht.) collected from Than, Dist. Rajkot in the month of December & March 2016 before & after flowering respectively. A Herbarium was prepared and authenticated by Dr. Kunjal Soni and deposited in School of Pharmacy SOP/COG/473/2016.

Extraction

Stem, leaf and root of *M. sativa* were separated and shade dried. All three were then subjected to size reduction. 300 gm powder of stem, leaf and root were macerated separately with toluene for 24 hours at room temperature and extract was filtered with the help of muslin cloth, marc was then macerated for 24 hours at room temperature with chloroform then filtered with the help of muslin cloth. Subsequently same procedure repeated for methanol and water. Successive extraction procedure completed within 4 days. Extract were then subjected for evaporation on heating mantle below 40°C. All 12 extracts were subjected for evaporation. Subsequent drying was done on water bath.

Mobile phase development

Pilot TLC were developed for methanol extract using various mobile phases. After observing the pilot results, further TLC were developed by adding ethyl acetate for removal of tailing. The mobile phases used were Chloroform: Methanol 3:7, 1:3, 5:5, 3:1 and 7:3. Chloroform : Methanol 1:5, 1:7, 1:9, Chloroform : Methanol : Ethyl acetate 1:5:1, 1:7:1, 1:9:1, Chloroform : Methanol: Ethyl acetate 1:12:1 & 1:15:1, Chloroform : Methanol : Ethyl acetate 1:5:3, 1:7:3 and 1:9:3 and Chloroform : Methanol : Ethyl Acetate 1:7:5, 1:9:5 and 1:5:5.

HPTLC

HPTLC fingerprinting of methanolic extract was performed in Dept. of Pharmaceutical Sciences, Saurashtra University, using the mobile phase Chloroform: Methanol: Ethyl acetate (1:5:5), as it gave most appropriate TLC fingerprint, 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

Six 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

Eight peaks were detected at 254nm (Table 1, Fig. 2, 3, 4) and six peaks were detected at 366nm (Table 2, Fig. 5, 6, 7) upon HPTLC of methanolic extract of *M. sativa* roots using mobile phase Chloroform: Methanol: Ethyl Acetate (1:5:5).

Table 1. R_f & Area Under Curve of HPTLC of methanol extract of *M. sativa* root at 254nm

Peak	R_f	Area Under Curve	Area %
1	0.11	1223.1	9.39
2	0.21	359.0	2.76
3	0.35	1677.4	12.88
4	0.44	3234.8	24.84
5	0.61	644.6	4.95
6	0.62	582.5	4.47
7	0.68	699.6	5.37
8	0.86	4602.8	35.34

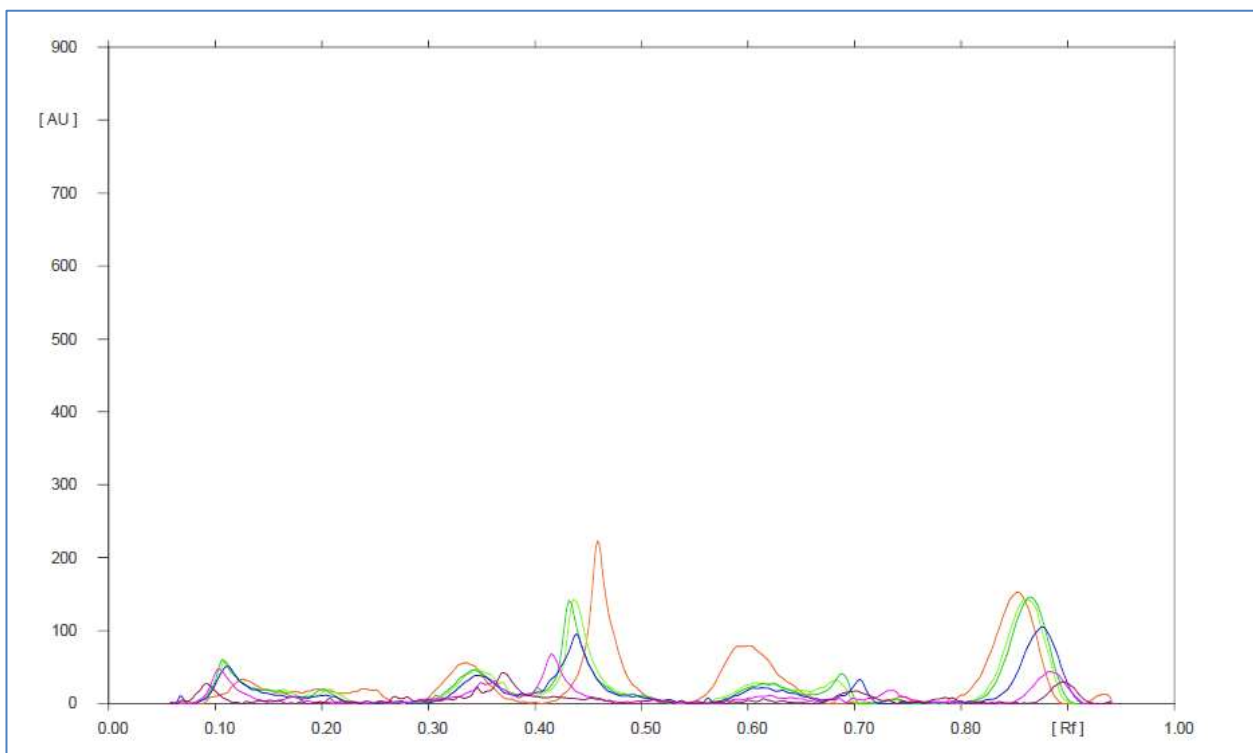


Figure 2: HPTLC 2D densitometric superimposable chromatogram of methanol extract of *M. sativa* root at 254nm (chloroform: methanol: ethyl acetate-1:5:5)

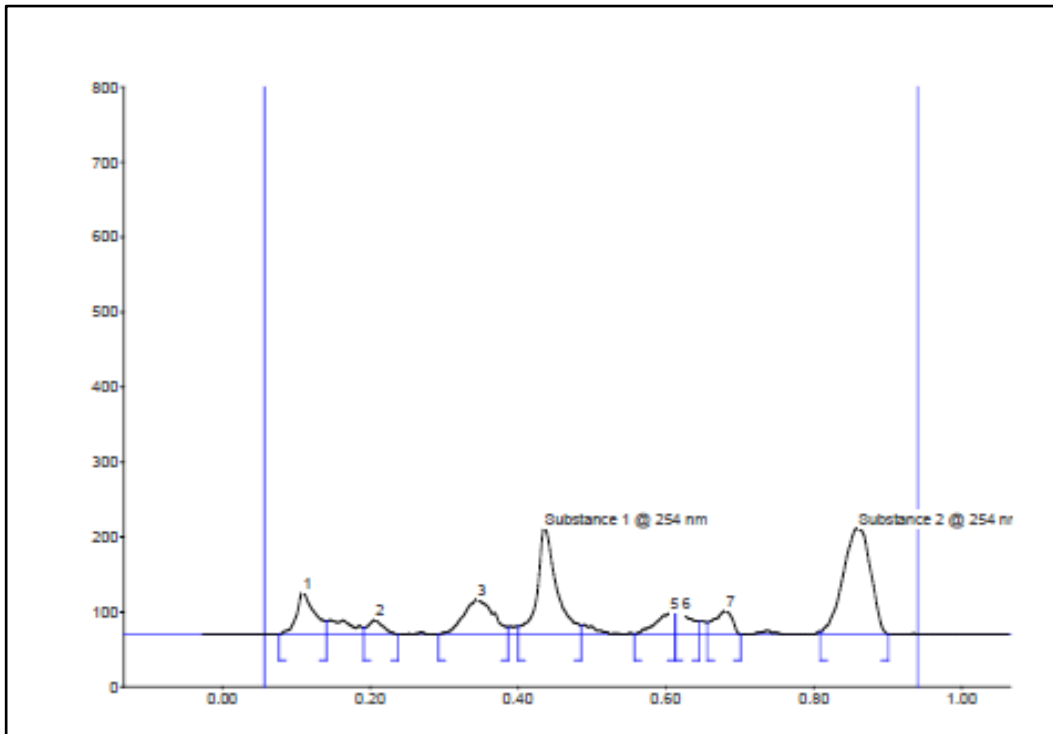


Figure 3: HPTLC chromatogram of methanol extract of *M. sativa* root at 254nm(chloroform: methanol: ethyl acetate-1:5:5)

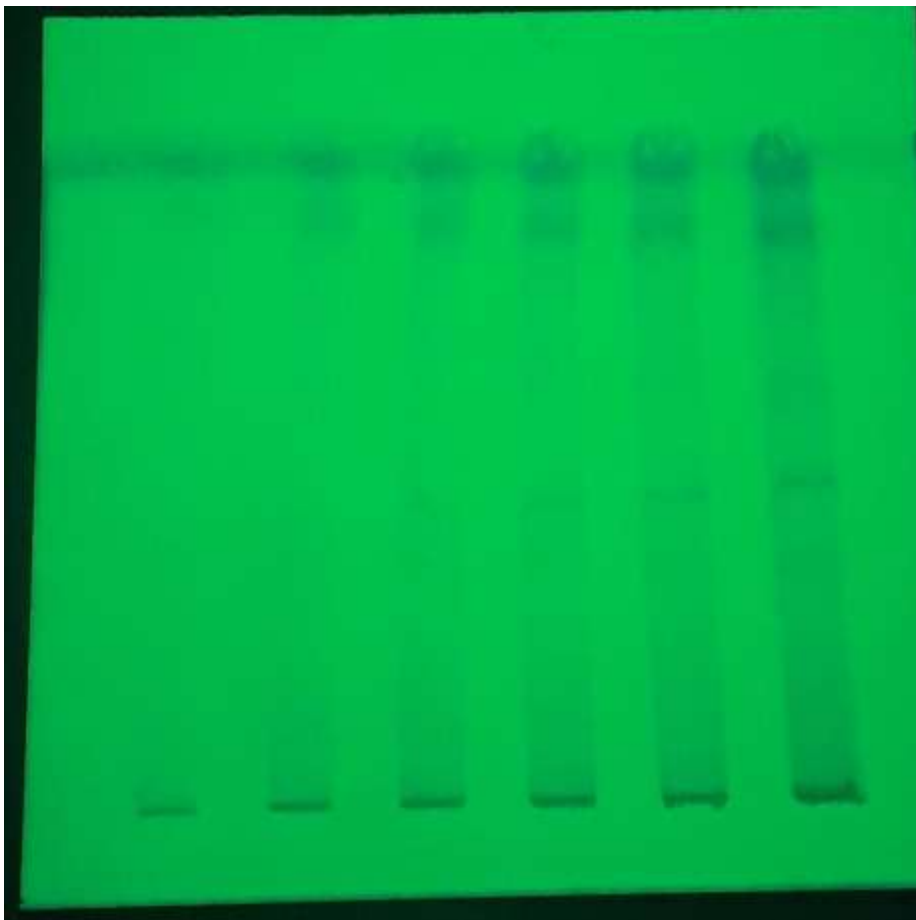
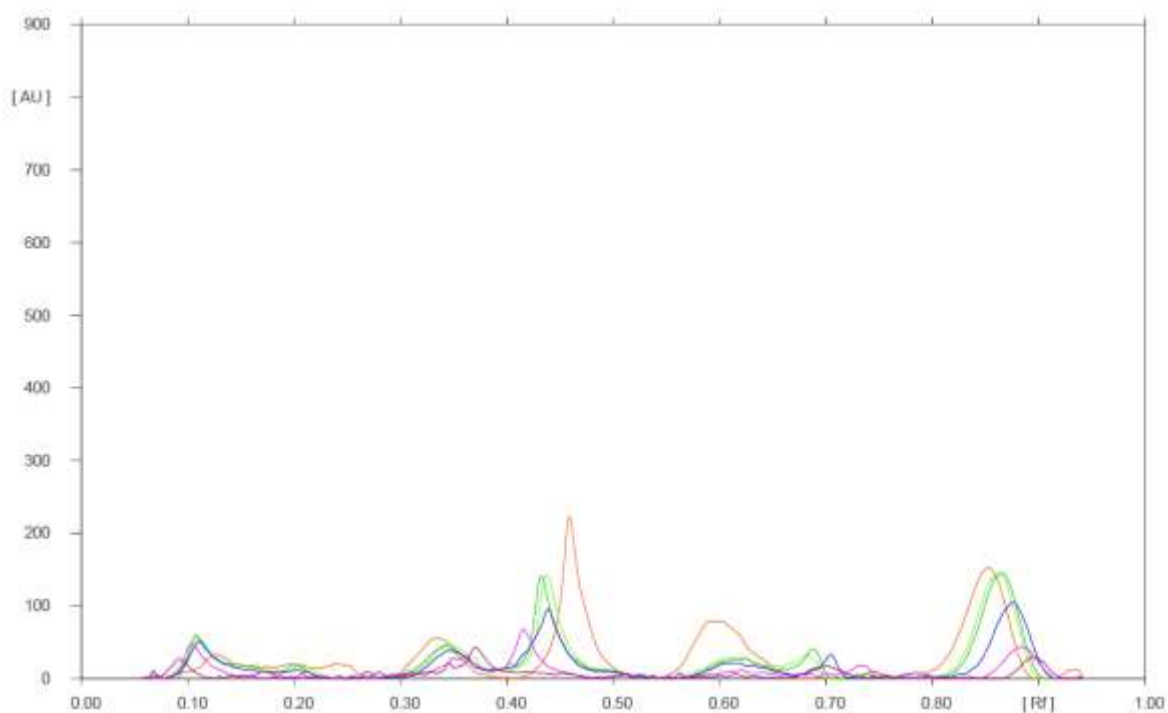


Figure 4: HPTLC Fingerprint of methanol extract of *M. sativa* root at 254nm(chloroform: methanol: ethyl acetate-1:5:5)

Table 2. R_f & Area Under Curve of HPTLC of methanol extract of *M. sativa* root at 366nm

Peak	Rf	Area Under Curve	Area %
1	0.11	800.6	25.82
2	0.20	193.5	6.24
3	0.40	108.1	3.48
4	0.43	1301.4	41.96
5	0.69	366.7	11.83
6	0.87	331.0	10.67

Figure 5: HPTLC 2D Densitometric superimposable chromatogram of methanol extract of *M. sativa* root at 366nm (chloroform: methanol: ethyl acetate-1:5:5)

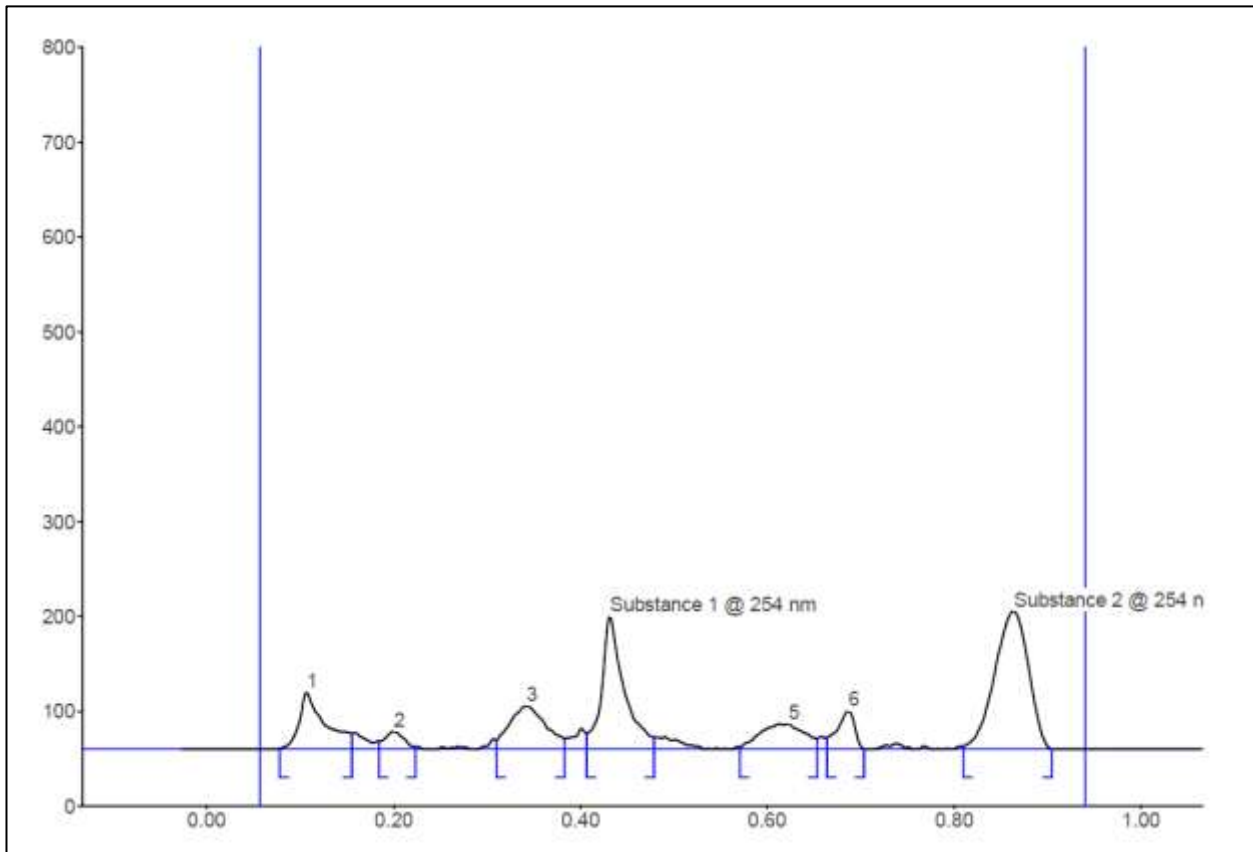


Figure 6: HPTLC chromatogram of methanol extract *M. sativa* root at 366nm (Chloroform: Methanol: Ethyl- acetate 1:5:5)

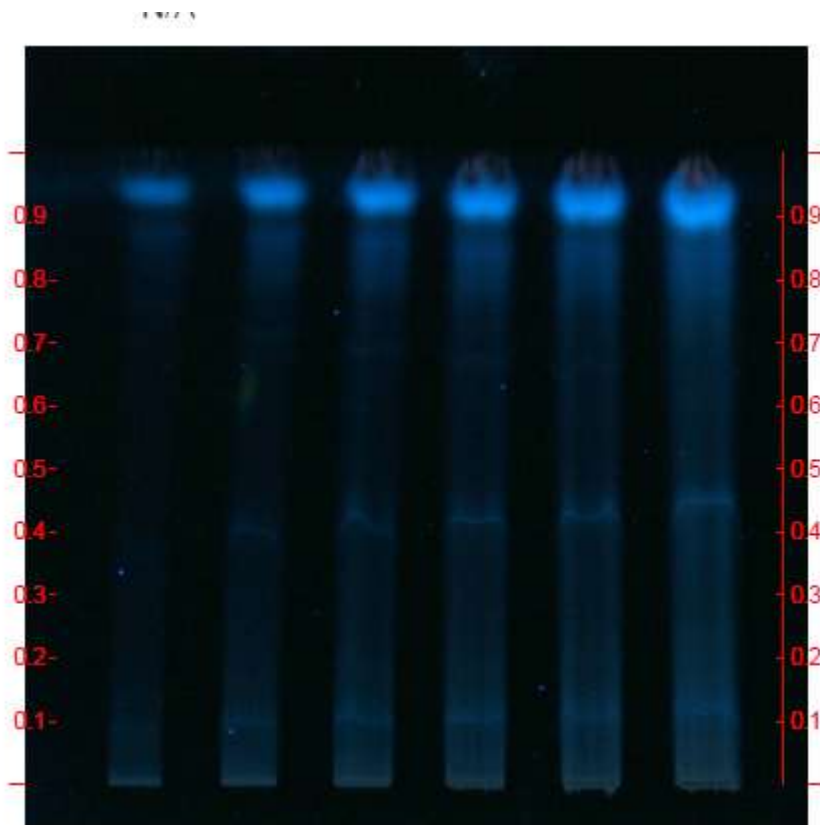


Figure 7: HPTLC Fingerprint of methanol extract of *M. sativa* root at 366nm(chloroform: methanol: ethyl acetate-1:5:5)

The present work can be helpful to herbal industry as an important standardization parameter of *M. sativa* roots, and especially its alcoholic formulations and extracts, since *M. sativa* roots are a part of several as they are indicated in a broad spectrum of diseases¹⁻⁷. This work can be specifically useful for authentication of raw material of the roots and in detection of adulteration, which will ultimately benefit the people who consume *M. sativa* root formulations.

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