

A Novel Approach of the Isolation of L-Dopa from the Methanolic Extract of *Mucuna pruriens* Seeds and its Quantitative Analysis by HPTLC.

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ABSTRACT

Mucuna pruriens (Fabaceae), also known as cowitch and velvet bean contains very powerful neurotransmitter precursor L-Dopa. The seeds of *Mucuna pruriens* accumulate 0.2%-2% L-Dopa in their dry weight. L-Dopa is an amino acid that converts into dopamine. Dopamine is an essential component of our body and it requires for the proper functioning of the brain. The seeds powder of *Mucuna pruriens* has long been used in traditional Ayurvedic Indian Medicine for hypertensive, hypoglycemic agent and Parkinson diseases. The research was focused on a novel approach of the isolation of L-Dopa in semi pure form by chemical fractionation followed by quantitative analysis of L-Dopa by HPTLC.

Key Words: *Mucuna pruriens*, L-Dopa, Isolation, HPTLC.

INTRODUCTION

Medicinal plants are playing an important role worldwide for their biological efficacy against various diseases and less side effects. *Mucuna pruriens* (Fabaceae), commonly known as velvet bean, is an annual climbing plant that grows 3-18m in height and indigenous of tropical regions, especially Africa, India and the West Indies. Its flowers are white to dark purple and hang in long clusters of pods which contain seeds known as *Mucuna* beans^{1, 2}. The seeds of velvet bean is a natural source of L-Dopa (L-3,4-dihydroxyphenylalanine) which has been reported for antiparkinson's, aphrodisiac, antidiabetic, maleinfertility and neuroprotective activities.^{3, 4, 5, 6}

Mucuna pruriens seeds in addition to levodopa contains, alkaloids mucunine, mucunadine, prurienidine, prurienine, trypsin, tryptamine, tyrosine, 5-hydroxytryptamine, histidine, cystine, fatty acids, glutamic acid, glutathione, glycine, isoleucine, lysine, methionine, nicotine, serine, serotonin, threonine.^{7, 8} The first attempt of the present study was, the isolation of L-Dopa from the methanolic extract of *Mucuna pruriens* seeds by fractionation method with above 92% of purity. Also TLC and HPTLC method was developed for preliminary and quantitative analysis of L-Dopa.^{9, 10}. The present paper is simple, accurate and reproducible for the isolation and the quantitative analysis of L-Dopa.

MATERIALS AND METHODS

Material: The seeds of *Mucuna pruriens* were purchased from the local market, Tamilnadu and it was authenticated by Botanist, A. V. V. M. Sri. Pushpam

College, Poondi 613503, Thanjavur District, Tamilnadu. Extraction and Fractionation was carried out using analytical grade solvents obtained from SD fine chemical limited (Mumbai). Reference standard L- Dopa purity of 99% from the Himalaya Drug Company (Bangalore). Analytical grade solvent Butanol, Acetic acid and Hydrochloric acid obtained from SD fine chemical limited (Mumbai) and precoated silica gel 60F254 TLC aluminum plates of uniform thickness of 0.2mm purchased from Merck (Mumbai).

Methods: Extraction with Methanol: About 1 kg of *Mucuna pruriens* seeds was subjected for size reduction to coarse powder and extracted by maceration with 4liters of methanol about 8hours at room temperature. Filtered through ordinary filter paper and the same process repeated for twice. Combine the filtrate and was concentrated using water bath at 70-80°C in the fume hood. Finally the soft mass was obtained, further it was taken for fractionation.

Isolation of L - Dopa from the methanolic extract of *Mucuna pruriens* seeds by fractionation: About 30g of methanolic extract was taken in 500ml round bottom flask and reflux with hexane (each300ml×3) at 40°C for 20minutes and hexane soluble part was separated through ordinary filter paper. Further hexane insoluble residue was reflux with chloroform (300ml×3) at 50°C for 20minutes and chloroform soluble part was separated through ordinary filter paper. Further chloroform insoluble residue was reflux with ethyl acetate (300ml×2) at 50°C for 20minutes and ethyl acetate soluble part was separated through ordinary filter paper. Then ethyl



Figure 1. (A) Isolated compound and (B). L -Dopa Standard

Table.1. R_f value of isolated compound and standard

Sample	R _f value	Color of the band
A	0.36	Brownish Pink
B	0.36	Brownish Pink

acetate insoluble residue was reflux with methanol (300ml×3) at 60°C for 15 minutes and methanol soluble part was separated through ordinary filter paper. Demineralize water 75ml was added to the methanol insoluble residue and was stirred gently using glass rod, during which the L- Dopa was settled down at the round bottom flask. Decanted the water soluble part without loss of L-Dopa and repeated the same process twice. Finally it was collected in whatman filter paper and rinsed with 25ml of methanol to increase the purity of L-Dopa further it was kept for drying in oven at 50°C for 20 minutes.

Chromatographic conditions: Chromatograph was performed on 10×10cm of precoated silica gel 60F254 TLC plate, application of standard and test sample at 25mm distance on the TLC plate. Then the plate was developed using optimized solvent system

Butanol:Acetic acid:Water(80:20:20), as a mobile phase^{11, 12} in a glass chamber, previously saturated for 30 minutes. The Plate was developed up to 8cm and the average development time was 60 minutes. After development the plate was air dried for 10 minutes and sprayed with ninhydrin reagent^{11, 12} (100mg of ninhydrin in 100ml of methanol). The plate was dried at 105°C for 5 minutes to enable the full color of the spot to develop.

HPTLC profile of L - Dopa: High Performance Thin Layer Chromatography technique is widely employed in pharmaceutical industry in identification and detection of adulterants in herbal product, for simultaneous assay of several components in a multi-component formulation and in quality control of herbs and health foods.

Preparation of standard solution: The 10mg of standard L-Dopa was dissolved in 100ml of 10% hydrochloric acid in water and sonicated for 5 minutes.

Preparation of test solution: The 10mg of isolated compound was dissolved in 100ml of 10% hydrochloric acid in water and sonicated for 5 minutes.

Instrumentation: HPTLC system equipped with a sample applicator linomat V, Camag TLC (Thin layer chromatography) scanner III, Camag reprostars III and Wincats software were used.

Quantitative estimation of L-Dopa by HPTLC: The equal concentration of 10µl of both test sample and standard were applied on the TLC plate at 25mm distance by a Camag HPTLC system equipped with a sample applicator linomat V. Then the plate was developed using optimized solvent system, Butanol:Acetic acid:Water (80:20:20), as a mobile phase in a glass chamber, previously saturated for 30 minutes. Three identical plates were prepared for concurrent results. The Plate was developed up to 8cm and the average development time was 60 minutes. After development, the plate was air dried for 10 minutes and sprayed with ninhydrin reagent. Then the plate was dried at 105°C for 5 minutes to enable the full color of the spot to develop. Further the plate was scanned using Camag TLC scanner III at 600nm wavelength in absorbance mode (W lamp) equipped with Wincats software. The

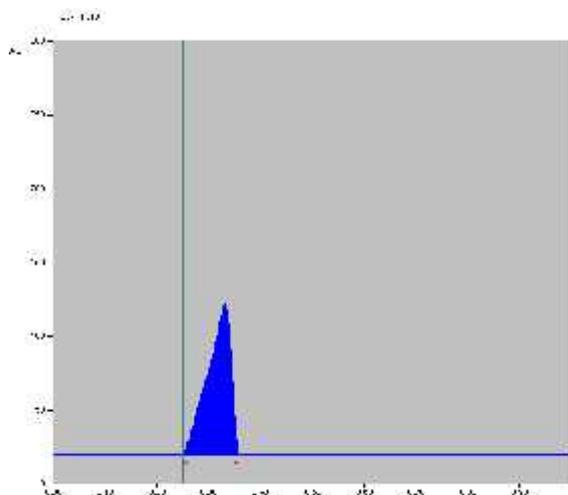


Figure 2. HPTLC Chromatogram of isolated compound

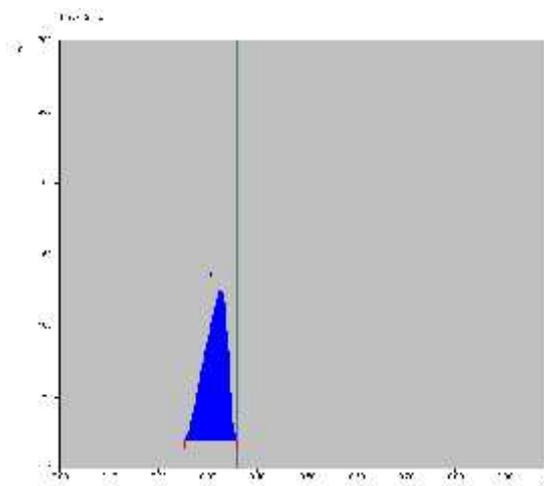


Figure 3. HPTLC Chromatogram of standard (L-dopa).

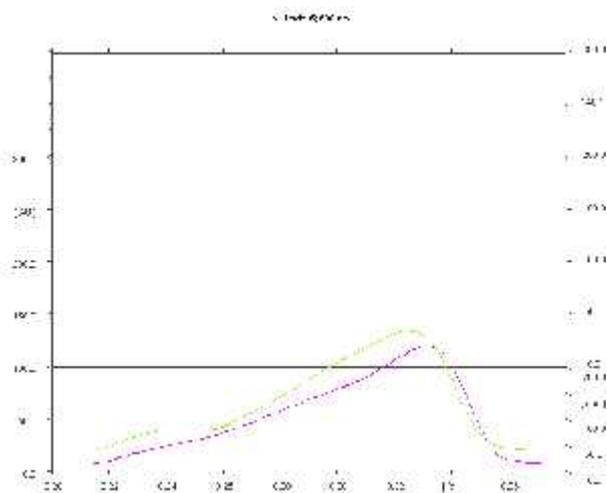


Figure 4. Spectral comparison of isolated compound & Standard (L-dopa) Scanned at 600nm.

purity of L-Dopa was quantified by comparing the R_f value and peak area of the spectrum with that of the standard.

Calculation: Determination of L- Dopa was calculated by the following formula;

$$\text{Area of the Isolated Compound} \times \text{Standard (99\%)} \div \text{Area of the standard}$$

RESULTS AND DISCUSSION

The work carried out on this plant was mainly on the method of extraction followed by isolation of L-Dopa by fractionation method using non polar to polar solvents (Hexane, Chloroform, Ethyl acetate, Methanol, Water). Extraction of *Mucuna pruriens* seeds with methanol gave the sticky solid extract and the yield was obtained 3% From this crude extract L-Dopa was isolated about 92% of purity by fractionation method and the compound yield was obtained 1.6% on the basis of extract weight.

Thin layer chromatography was performed through the test sample with respect to the reference standard L-Dopa, purity of 99% using optimized solvent system Butanol:Acetic acid:Water (80:20:20), as a mobile phase. After development the plate was air dried for 10 minutes and sprayed with 0.1% ninhydrin in methanol. The plate was dried at 105°C for 5 minutes. The brownish pink color spot was obtained and the TLC pattern revealed that the profiles are similar (Figure.1) with respect to the standard L-Dopa having the R_f value of 0.36 (Table.1).

Quantitative determination of L-Dopa in the test sample with respect to the standard was performed by HPTLC. The derivatized TLC plate was scanned using camag TLC scanner III at 600nm wavelength in absorbance mode (White lamp) equipped with wincats software. Under the above described conditions high resolution spectrum was obtained for L-Dopa. The confirmation of L-Dopa in the test sample was evaluated by comparing the R_f value and peak area of the spectrum with that of the standard. Further the purity of L- Dopa in the test sample was calculated by comparing the peak area with respect to the standard. From the obtained spectrum the peak area of L-Dopa (isolated compound) was 4170.5 Area under the

curve (AUC) Fig.2 and for the standard 4478.2 AUC Fig.3. According to the standard peak area (L-Dopa), the purity of isolated compound was calculated as follows;

$$\text{Purity of L-Dopa} = \frac{4170.5 \times 99}{4478.2}$$

Purity of L-Dopa in the isolated compound = 92.2%

CONCLUSION

On the basis of the results the present study was concluded that the purity of about 92% L-Dopa was isolated from the methanolic extract of *Mucuna pruriens* seeds by the fractionation method. The L-Dopa thus obtained can be further identified and characterized using TLC and HPTLC. Purity of L-Dopa was concluded from the HPTLC spectral data. This article can be useful for the isolation of L-Dopa in lab scale with less time consuming and the analysis of L-Dopa in the Herbal drugs by HPTLC Method.

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Conflict of interest

Conflict of interest declared none.

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