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Isolation and estimation of L-Dopa in two species of Mucuna: *Mucuna pruriens* and *Mucuna prurita*

S.Raghavendra[1], V.Kumar[1], M.H.Moinuddin Khan[2]*, C.K.Ramesh[3]

[1]Department of Biochemistry, P.G Center, Shivagangothri, Kuvempu University, Tholahunsae, Davangere-Karnataka, (INDIA)

[2]Department of Chemistry, Jawaharlal Nehru National College of Engineering, Shimoga-577 204, Karnataka, (INDIA)

[3]Department of Biotechnology, Sahyadri Science College, Shimoga-577 203, Karnataka, (INDIA)

Tel: +91-8182-276707; Fax: +91-8182-222450

E-mail: raghu_rsn2004@yahoo.co.in, E-mail: moin_nallur@rediffmail.com

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ABSTRACT

Mucuna pruriens and *Mucuna prurita* belong to the family *Papillonaceae*. The seeds and leaves of these plants contain large amount of L-Dopa. L-Dopa[L,3,4- Dihydroxy phenylalanine] is one of the most widely used drugs in the treatment of Parkinson's disease. The present paper deals with the simple method for the isolation and quantitative concentration of L-Dopa in leaf, stem, callus and seeds of two *Mucuna* species. Qualitative analysis of L-Dopa was carried out by TLC and quantitative determination was done spectrophotometrically. The comparative analysis between two species reveal that L-Dopa concentrations are considerably higher in *Mucuna prurita* compared to *Mucuna pruriens* in all tissue types and seeds.

KEYWORDS

Callus;
L-Dopa;
Parkinson's disease;
Pruriens;
Qualitative.

INTRODUCTION

Mucuna pruriens and *Mucuna prurita* are also called as Velvet bean are tropical legumes belong to the family *Papillonaceae*. These are twiners with trifoliate leaves, purple flowers and pods covered with hairs. The two species differ from each other at their seed coat colour. The seeds of *Mucuna pruriens* bear black colour, *Mucuna prurita* seeds with white. The plants are said to contain large amount of L-Dopa[1-2]. Seeds of *Mucuna* have been described as a useful therapeutic agent in various diseases of the human nervous and reproductive system including Parkinson's disease[3]. In addition to L-Dopa the seed also contains serotonin, tryptamines, bufotenine and N, N-dimethyl tryptamine. These are structurally related to serotonin. Serotonin itself is a neurotransmitter in mammals and has a variety of effects on nervous and smooth muscle respiration, heart and cardiovascular system and gastro intestinal tract.

L-Dopa[L, 3, 4-Dihydroxy phenylalanine] is one of the most highly active alleo chemicals. L-Dopa is produced via oxidation of tyrosine by the copper containing enzyme tyrosinase in the presence of molecular oxygen[4], which can be converted into neurotransmitter, dopamine and the important hormones, adrenaline and noradrenalin[5]. L-Dopa is also an essential precursor in the biosynthesis of several phenyl propanoids and melanin[4].

Parkinson's disease is a degenerative, neurological

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disorder[6]. The very first description and treatment of Parkinson's disease was found in Ayurveda. Numerous factors including reactive oxygen species induced damage exotoxicity; mitochondrial dysfunction and inflammation mediated cell injury are considered in the etiology of this disorder. The major drug in the treatment of Parkinson's disease is L-Dopa, the physiological amino acid precursor of dopamine[7]. Legumes are reported to produce L-Dopa, amongst *Mucuna* seeds are the richest source. In the present investigation we describe the isolation and estimation of L-dopa from two mucuna species.

EXPERIMENTAL

The present work is concentrated on the isolation, separation and estimation of L-dopa in the two species of Mucuna, *Mucuna pruriens* and *Mucuna prurita* one that produces black seeds and the other white. The leaf, stem, seeds of both the variety and callus of stem and leaf were used in our work. The plants were cultivated in the botanical garden of Sahyadri Science College and were used for the concerned work. All the chemicals and reagents used were of analytical grade and procured from Sd fine Chemicals and Hi-Media, Mumbai.

Reagents used for the identification of L-Dopa were silica gel, butanol, acetic acid, and ninhydrin. For the estimation of L-Dopa the reagents used were 0.5N hydrochloric acid, nitrate molybdate reagent(dissolve 10gm of sodium nitrate and 10gm of sodium molybdate in 100cc of distilled water), 1N sodium hydroxide and standard solution of L-Dopa(1 mg/ml.). Callus culturing was done on Murshige Skoog media[MS media]. The hormones used for the induction of callus were Auxin–IAA[Indole -3 acetic acid]-2mg/ltr, BAP[6 -Benzyl amino purine]-0.2mg /ltr.

The instruments used were centrifuge, spectrophotometer, TLC plate, laminar airflow[LAF] and incubation chamber.

Establishment of callus cultures

The explant materials were properly sterilized before inoculation. About 2-4 sterile explants (leaf and stem of both verities) were transferred to each culture vessel containing MS medium supplemented with growth regulators. The inoculated culture vessels were then transferred to incubation chamber. The culture vessels were incubated at 25 ± 2^0C. Observations were recorded after 5 days of incubation and at regular intervals thereafter.

Isolation of L-dopa

1gm of explant was taken and crushed using pestle and mortar by adding 10cc of distilled water. The extract was boiled for 10min and cooled. Extract was centrifuged for about 10 min at 5000rpm. The supernatant was collected, boiled and cooled. Again the supernatant is centrifuged and the resulting supernatant after centrifugation was collected which was used for the estimation. 1cc of extract was taken in 10ml volumetric flask and the volume was made up to 10cc with distilled water. Same methodology was followed for isolation of L-Dopa from leaf, stem, seed and callus of leaf and stem[8-9].

Qualitative analysis of L-Dopa by thin layer chromatography

Isolated samples were applied along with standard L-Dopa on TLC plate, made of silica gel (0.25mm). The plates were kept in TLC chamber containing the developing solvent (water, acetic acid, and butanol-5:4:1). The separated L-Dopa was identified by spraying ninhydrin reagent which forms purple colored spots. The L-Dopa present in leaf, stem, seeds and callus was identified by comparing with the standard L-Dopa.

Spectrophotometric estimation of L-Dopa

1cc of unknown sample and 1cc of standard was taken in separate test tube and for blank 1cc of water was taken. Reagents were added in the given order, mixed well after each addition. 1cc of 0.5N hydrochloric acid, 1cc of nitrate molybdate reagent[a yellow color results at this point] and 1cc of 1N sodium hydroxide[a red color results]. Optical density was recorded at 520 nm using ELICO SL-157 spectrophotometer (ELICO, India).

RESULTS

Spectrophotometric estimation of L-DOPA

Concentration of L-Dopa in the unknown samples

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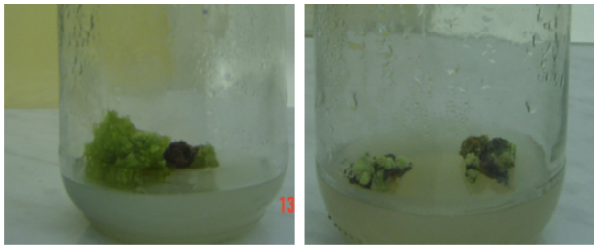
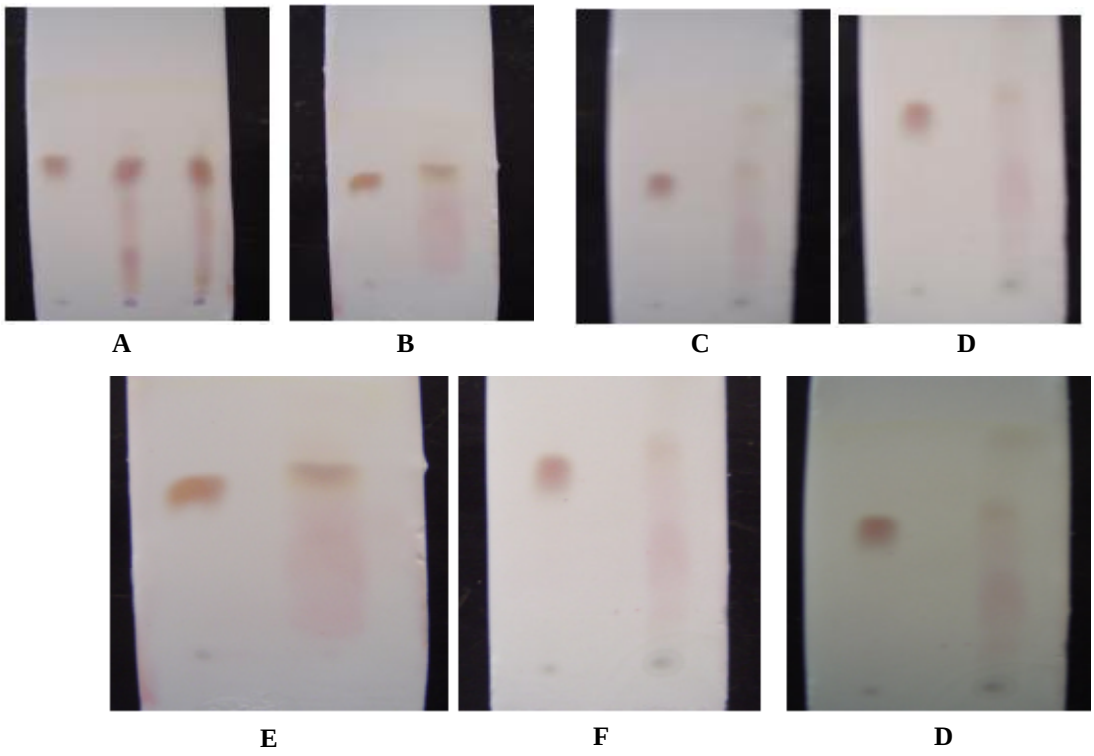


2mg/ltr IAA and 0.2mg/ltr BAP hormone concentration was used for the establishment of callus. At this concentration there was induction of callus both in leaf and stem; Photograph A – Showing the induction of callus in stem, Photograph B – Showing the induction of callus in leaf.

TABLE 1 : Spectrophotometric readings of the various explants used for the experiment

Plant parts used	O D at 520nm	Concentration in mg/g
	Mucuna pruriens	
Leaf	0.010	0.7 mg
Stem	0.030	2.0 mg
Leaf Callus	0.040	2.7 mg
Stem Callus	0.050	2.6 mg
Seed	0.070	4.6 mg
	Mucuna prurita	
Leaf	0.020	1.3 mg
Stem	0.030	2.0 mg
Leaf Callus	0.060	4.0 mg
Stem Callus	0.050	3.3 mg
Seed	0.090	6.0 mg



Separation of L-Dopa was done by TLC separation method. L-Dopa is present in all the explant used for the separation (leaf, stem, callus and seeds of two species of *Mucuna)*. Here standard L-Dopa was used to identify the presence of L-Dopa in all the explants. Separate plating was done for each explants used. The first spot in each plate represent the standard DOPA. The photograph clearly shows the presence of L-Dopa in all the explants;
Photograph -(A) Presence of L-DOPA in two varieties of seeds - *Mucuna pruriens* and *Mucuna prurita*, (B) Presence of L-DOPA in *Mucuna prurita* of callus, (C) and (D) Presence of L- DOPA in *Mucuna prurita* of stem and leaf, (E) Presence of L-DOPA in *Mucuna pruriens* of callus, (F) and (G) Presence of L-DOPA in *Mucuna pruriens* of stem and leaf.

can be calculated by using the following formula. "OD of test/OD of standard X concentration of standard X dilution factor"

The dilution factor is 20, because 1gm of the explant was crushed in 10cc water and from this 1cc was taken and made to 10cc and from this 1cc was taken and used for estimation. The concentration of standard is 1, as 1mg/cc concentration of standard L-Dopa was used.

The following table shows the concentration of L-Dopa in the various explants used, by substituting the OD values in the above formula.

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L-Dopa was quantified by spectrophotometric method[10]. The amount of L-dopa present in each of the explant used is given in TABLE 1. It clearly indicates that, seeds of the plant have the highest amount of L-Dopa, compared to the callus, stem and leaf of both verities. *Mucuna prurita* seed has more amount of L-Dopa than *Mucuna pruriens*. Stem of both the species of plants have more amount of L-Dopa than compared to leaf. Comparing to the leaf and stem, callus of the leaf and stem has more amount of L-Dopa.

DISCUSSIONS

Mucuna pruriens and *Mucuna prurita* are twinners with trifoliate leaves contain large amount of L-Dopa; the physiological amino acid precursor of dopamine is the major drug in the treatment of Parkinson's disease. TLC separation indicates the presence of L-Dopa in stem, leaves, seed and callus cultures of both the varieties of the plant and also indicates the simple methodology of separation. Spectrophotometric estimation is a quantitative determination of L-Dopa based on the observation that L-Dopa reacts with nitrate molybdate reagent to give a compound having yellow color, this color changing to red on addition of excess of alkali.

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