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Research Article

Comparison of *l*-Dopa Content in Three Species of Genus *Mucuna* by Different Extraction Techniques

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ABSTRACT

In the present study, attempts are made to develop suitable method(s) for extraction of L-DOPA from the powdered seeds of 3 species of *Mucuna* using different solvents and conditions. The seed powder of both plants was subjected to 6 different extraction methods, with different solvent ratios. All the extracts were analyzed using RP-HPLC and was validated according to The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines. The L-DOPA extraction was best with Methanol Water mixture in a cold maceration technique and overall gives good extraction efficiency in all the three plants giving concentrations of 5.03%, 13.36 % and 16.78% of L-DOPA in *Mucuna gigantea*, *Mucuna pruriens* and *Mucuna monosperma*, respectively. The present investigation was done to study the extraction efficiency of various extraction methods of L-DOPA content in seed extracts of *Mucuna* and compare it.

Keywords: Comparison, Extraction efficiency, HPLC, L-DOPA, *Mucuna*.

INTRODUCTION

L-(3,4-dihydroxyphenyl)-L-alanine(L-DOPA) is a precursor to many neurotransmitters like dopamine, norepinephrine (noradrenaline), and epinephrine. L-DOPA crosses the Brain Blood Barrier whereas, dopamine cannot. In the Central Nervous System, L-DOPA converted into dopamine by the enzyme aromatic L-amino acid decarboxylase, also known as DOPA decarboxylase (DDC)[1].

Various *Mucuna* species have been studied in developing countries as cover crops for food self-sufficiency development and soil fertility improvement; furthermore, their bioactive substances have been thoroughly evaluated, particularly L-DOPA[2]. In contemporary medicine, *Mucuna* remains a genus of interest since its L-DOPA content and use in treatment of Parkinson's disease continues to be evaluated in biochemical research[3].

Mucuna pruriens is commonly known as velvet bean, cowitch, cowhage,etc. *Mucuna pruriens* has long been used in traditional Ayurvedic Indian medicine for many diseases[4]. The seeds are noted to be a natural source of L-DOPA and are also used as a substitute for the synthetic L-DOPA[5]. It is also known to show wonders as antiparkinson's, aphrodisiac, antidiabetic, male infertility and neuroprotective activities[4,6–8].

Mucuna monosperma is commonly known as Negrobean & periyattalargai[9]. Its seed materials have lately received more attention. Crude proteins, crude lipids, ash and nitrogen free extractives constituted 30.62%, 9.03%, 5.99% and 42.79%, respectively have been reported in the seeds. The seeds are also rich in minerals like potassium, calcium, magnesium and iron[9]. Anti-nutritional substances like total free phenols, tannins, and L-DOPA were also investigated[10].

Mucuna gigantea, also known commonly as Seabean, or in Hawaiian as Kāe'e, is indigenous to the Hawaiian Islands. Their pods show a range of color from green to brown and has hair-like structure called trichomes on it[2]. These seeds have high amounts of crude protein, crude fat, total free phenols and tannins when compared to other legumes. Researchers have showed that the amount of L-DOPA can be reduced to a great extent just by cracking and rinsing the seeds under running water, proving that L-DOPA is very much present in the seeds[2].

MATERIAL AND METHODS

Table 1: Method validation parameters.

Parameters	Values
Linearity	100-700 ppm
Correlation coefficient	0.992
Accuracy (Standard Addition Method)	98.83% recovery
Presicion	
Intraday	0.189
Interday	0.754
Limitof Detection(LOD)	2 ppm
Limit of Quantitation (LOQ)	5 ppm
Stability	Assay not decreased below 8%

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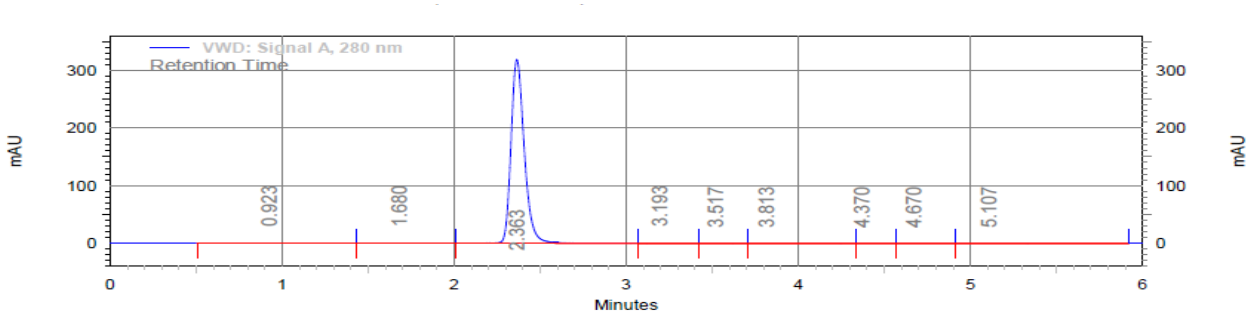

Figure 1: Chromatogram of the Standard.

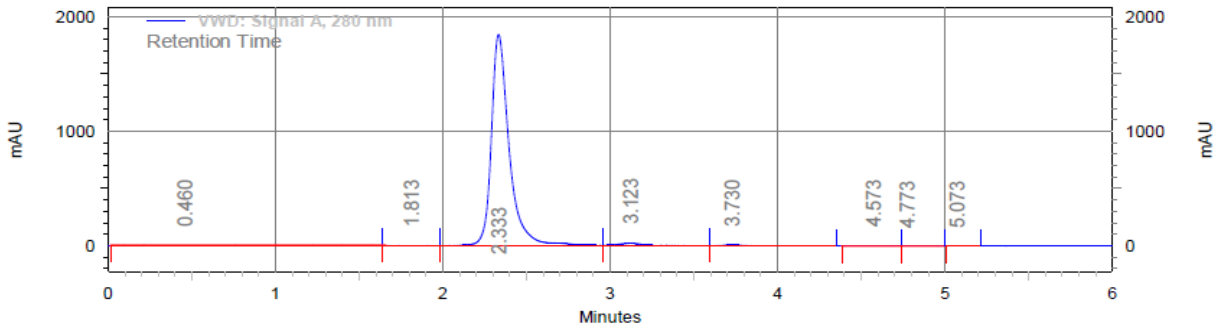

Figure 2:Chromatogram of Method 4 for *Mucuna prureins.*

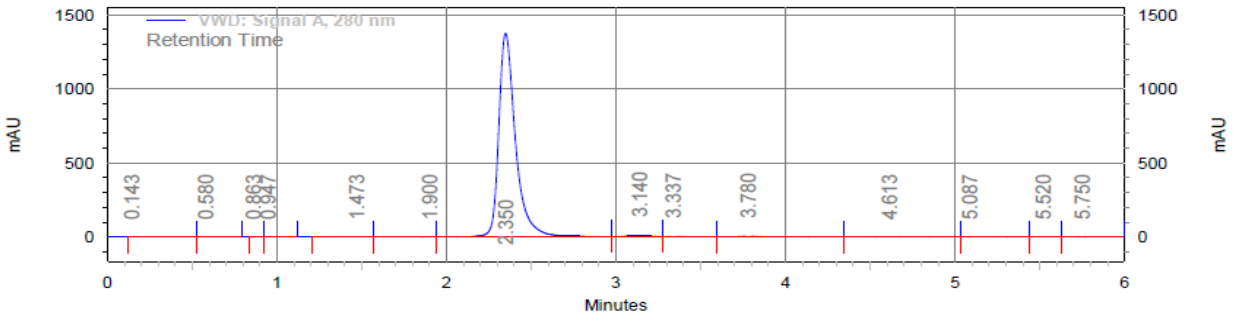

Figure 3:Chromatogram of Method 4 for *Mucuna monosperma.*

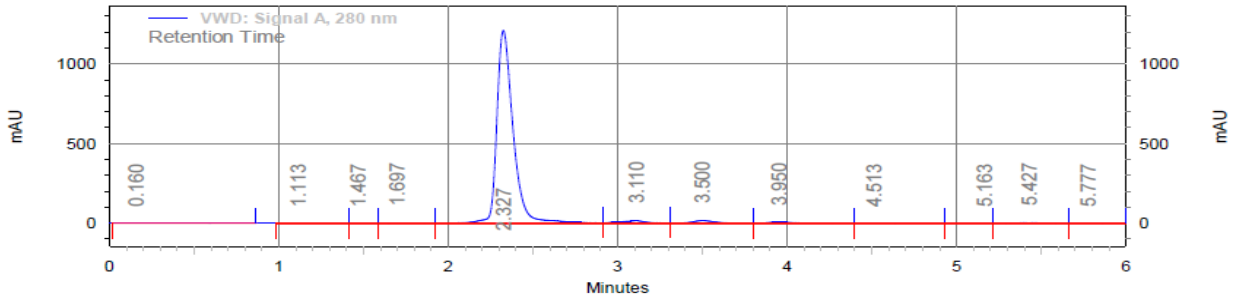

Figure 4:Chromatogram of Method 4 for *Mucuna gigantea.*

Collection and preparation of Sample

Mucuna gigantea seeds were received from The Botanic Reserves of the Cairns Regional Council, Australia. *Mucuna prureins* pods were collected from Sanjay Gandhi National Park, Borivali; Mumbai, India on February 2015 and *Mucuna monosperma* pods were collected from the forest area of Ganeshgudi, Dandeli; Karnataka, India. The pods were dry roasted so as the burn the external itchy trichomes and it also facilitated in opening the pods with ease. The seeds were collected and were kept in hot air oven at 40°C for checking its water loss. The Dried seeds were powdered using a grinder and passed through a sieve to achieve fine powder.

Preparation of Standard

99.9% pure L-DOPA standard was obtained from Pallav Chemicals and 1000 ppm standard stock was prepared[11].

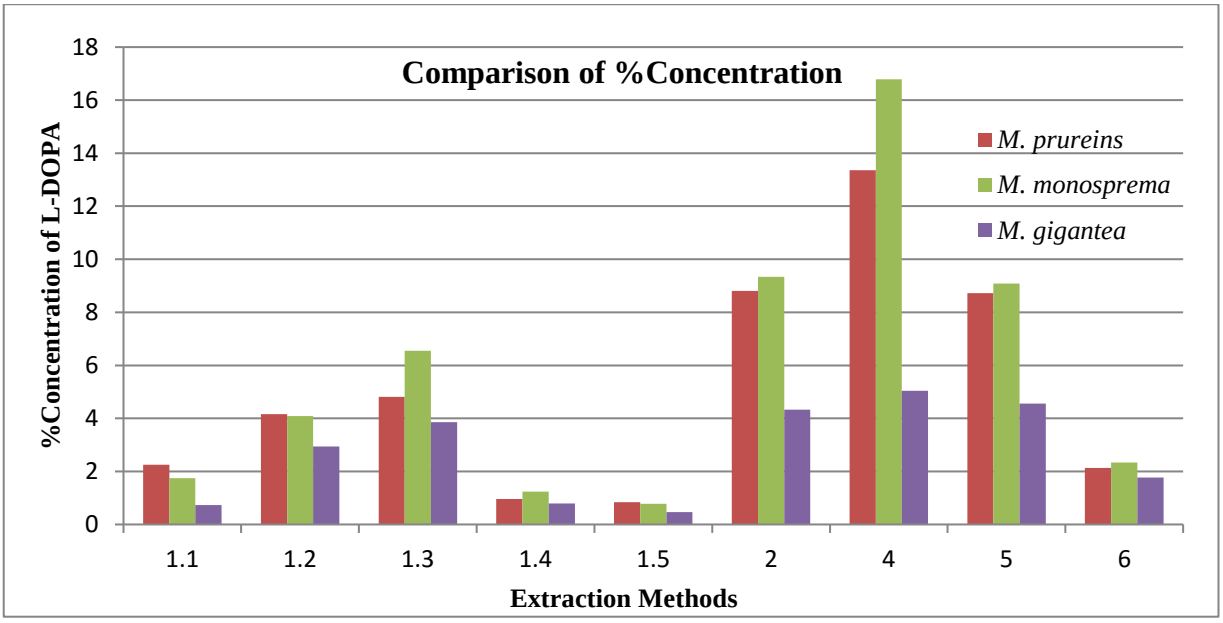

Figure 5: Comparison of extraction efficiency of L-DOPA from all three selected species of Genus *Mucuna*.

Chromatographic conditions and instrumentation

Chromatographic separation was performed with AGILENT HPLC (Model no. 1220 Infinity) equipped with binary pump and auto injector (20µl). OpenLabCDS Version A.04.06 chromatographic software wasused for data acquisition. Kromasil100–5–C18 (250mm× 4.6mm × 5µ); Part / Serial No: M05CLA25/E117509column was used for analysis. Mobile Phase used wasWater / Methanol / AcetoNitrile (100:60:40) (v/v) containing 0.2% Triethylamine, pH = 3.3 was filtered through 0.45 micron membrane filter (Millipore) and degassed by sonication; flow rate of 1 ml / min was maintained throughout the run. Column effluent was monitored at 280 nm with variable wavelength UVdetector[11].

Method Validation

Validation of the HPLC method was carried out as perICH guidelines.[12] Parameters such as Linearity, Accuracy, Precision, LOD and LOQ were taken up astests for analytical method validation and the values arelisted in table 1.

Preparation of the Plant extracts

Extracts were made using various techniques as listed below:

Method 1:[13]

This method was proposed by Takashi et. al., 2011[13]. The preparation of the sample remained the same and onlythe solvents used for the extraction have been changed soas to check the extraction efficiency of the sameprocedure with different concentration. The various solvent systems used were:

Method 1.1: acetonitrile:water:formic acid (80:20:1)
Method 1.2: acetonitrile:water (50:50),
Method 1.3: acetonitrile:water:formic acid (50:50:1),
Method 1.4: acetonitrile:formic acid (100:1),
Method 1.5: acetonitrile:water (80:20)

Method 2:[14]

The seed powder was defatted with acetone and thensuspended in water: ethanol (1:1) with 0.1 %ascorbic acid for 3 overnights. This was performedwith regular change of solvents.

It was diluted 1:100 by using water: ethanol (1:1) with 0.1% ascorbic acid for HPLC analysis.

Method 4:[15]

The seed powder was suspended in Water: Methanol (50:50) (v/v) and let it stand for 2 hrs unlike theoriginal method.

Method 5:[16]

In this method, heat reflux was done for the seed powder using 0.1N HCl solution.

Method 6:[17]

The seed powder was treated with water: ethanol 30:70, kept in tightly closed container for 7 days.

The supernatant was separated.

RESULTS AND DISCUSSIONS

The HPLC method discussed in the present work provides a convenient and accurate way for analysis of L-DOPA in three species of *Mucuna*. The retention time of standard L-DOPA is 2.363 mins as shown in fig. 1. As shown in fig. 2, 3 and 4, respectively, *Mucuna pruriens* extract shows retention time of 2.333 mins, *Mucuna monosperma* extract shows retention time at 2.350 mins and *Mucuna gigantea* extract shows retention time 2.327 mins. The match in the retention time confirms the presence of L-DOPA in all three selected species of *Mucuna*.

For quantitation purposes all the plant extract were made in triplicates and tested by HPLC. The Area under the Curve/ peak area was considered and used for calculations. The Formulae used were as follows[18]:

$$Response\ factor = \frac{Peak\ Area}{Standard\ Amount}$$

$$Amount\ of\ Unknown\ in\ the\ sample = \frac{Peak\ Area}{Response\ Factor}$$

$$\%\ Content = \frac{C \times V \times D}{10000 \times W}$$

Table 2: Mean Values of percent concentration of L-DOPA in the three selected species of Genus *Mucuna*

Method	*M. prureins* (%)	*M. monosprema* (%)	*M. gigantean* (%)
1.1	2.257	1.74	0.726
1.2	4.157	4.088	2.936
1.3	4.806	6.545	3.864
1.4	0.958	1.234	0.79
1.5	0.843	0.78	0.466
2	8.81	9.333	4.334
4	13.362	16.78	5.038
5	8.722	9.084	4.564
6	2.134	2.337	1.772

Where,
C= conc in mg/L
D = dilution factor
V = final total volume
W = Weight of the sample taken in g

In proposed method, Linearity was observed in the concentration range of 100–700 ppm. The mean values of L-DOPA content in the seed powder extracted by each of these methods are compiled in the Table 2 and Figure 5. *Mucuna gigantea* showed relatively low concentration of L-DOPA in comparison with other two plants for all the methods. Method 2 and Method 4 were good methods in terms of its extraction efficiency where all the three plants show approximately, 4%, 8% and 9% L-DOPA in *M. gigantea,M. pruriens* and *M. monosperma*, respectively. The L-DOPA extraction was best by Method 4 i.e. with Methanol Water mixture in a cold maceration technique and overall gives good extraction efficiency in all the three plants giving concentrations of 5.03%, 13.36 % and 16.78% of L-DOPA in *Mucuna gigantea, Mucuna pruriens* and *Mucuna monosperma*, respectively. Method 1.4 and 1.5 showed the least concentration of L-DOPA in all the three selected species.

CONCLUSION

Use of suitable extraction methods will increase versatile utilization of these seeds with high levels of bioactive compounds for the management of chronic diseases like Parkinson's. The present investigation suggests Water-Methanol system to remain the best solvent from all the solvents used for maximum extraction of L-DOPA for all the three species. According to literature a lot of work is done on *M. prureins* and its L-DOPA content; through this present study we can conclude *M. monosperma* to be a better source of L-DOPA.This will further help standardize procedures for extraction of L-DOPA from *Mucuna* plants and make a natural medicine against the symptoms of various diseases. However, for industrial application purposes, further investigations are required to develop mathematical model to control and predict the optimization parameters of the extraction process.

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