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Comparison of extraction efficiency of various methods to extract L-DOPA from *Mucuna pruriens* (L.) DC.

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Abstract: *Mucuna pruriens* seeds are noted to be a natural source of L-DOPA and are also used as a substitute for the synthetic L-DOPA. In the present study; attempts are made to develop suitable method(s) for extraction of L-DOPA from the powdered seeds of *Mucuna pruriens* using different solvents and conditions. The Seed powder was subjected to 7 different extraction methods and Method 1 was subjected to various solvent concentrations. Some methods used de-fatting procedure, either the method was cold maceration or in high temperature. Soxhlet extraction was also used in one of the extraction methods. All the extracts were analyzed using RP-HPLC. Mobile Phase used was Water: Methanol: AcetoNitrile (100:60:40) (v/v) containing 0.2% Triethylamine, pH = 3.3 and monitored at 280 nm with variable wavelength UV detector. The extraction was best with Methanol Water mixture in a cold maceration technique and overall gives good extraction efficiency of 13.36 % L-DOPA and id the best method giving highest extraction efficiency. The De-fatting method was the 2nd best methods giving approximately 8.8% L-DOPA and Method 5 viz, heat reflux method gives 8.7% L-DOPA making it the 3rd best method. There are not many studies done for optimization of extraction technique for L-DOPA despite an extensive work is reported for isolation, identification and pharmacological activities of L-DOPA from various plant sources. Keeping this in view, present investigation was done to study the extraction efficiency of various extraction methods of L-DOPA content in seed extracts of *Mucuna pruriens* and compare it.

Key words: Comparison; Extraction; HPLC; L-DOPA; *Mucuna pruriens*.

Introduction

L-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA) is a precursor to many neurotransmitters likedopamine, 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) (Simuni and Hurtig, 2008).

Mucuna pruriens, commonly known as velvet bean, cowitch, cowhage, Kawanch, kapikachu, nescafe, and sea bean is an annual climbing plant that grows 3–18m in height and originating from Africa, India and the West Indies. It has are white to dark purple flowers and hang in a long cluster of pods containing seeds known as *Mucuna* beans (Taylor, 2005). The seeds are noted to be a natural source of L-DOPA and are also used as a substitute for the synthetic L-DOPA (Kuber and Thaakur, 2007). It is known to be affective as antiparkinson's drug, aphrodisiac, antidiabetic, and neuroprotective agent. *Mucuna pruriens* seed in addition to levodopa, contains tryptamine, 5-hydroxytryptamine (5-HT), mucunine, mucunadine, prurienine and prurieninine and is rich in fatty content as well (Bala, *et al.,* 2004).

The extensive use of herbal medicines all over the world has made it very important to standardize the herbal formulations. One of the most important steps for getting the bioactive substances from the plant is extraction. Change in the method of extraction, solvents used, different extraction techniques can highly vary the quantity of the bioactive material extracted. Therefore, a suitable extraction method is important for obtaining the extracts with required pharmacological activities.

There is a heavy demand of *Mucuna pruriens* in the herbal drug market. Optimization of L-DOPA extraction and its quantification is important as ingestion of excessive amounts can lead to severe psychosis, nausea, emesis, arrthymia, hypertension and dyskinesias (Infante, *et al.,* 1990; Manini, *et al.,* 2001). There is a lack of studies done for optimization of extraction technique for L-DOPA despite an extensive work is reported for isolation, identification and pharmacological activities of L-DOPA from various plant sources. Keeping this in view, present investigation was doneto study the extraction efficiency of previously published extraction methods of L-DOPA content in seed extracts of *M. pruriens* and compares it (Dhanani, *et al.,* 2015).

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The B.P. describes a non-aqueous titration for the determination of L-dopa (British Pharmacopoeia, 1980). The U.S.P. recommends a non-aqueous titrimetric procedure with potentiometric end point determination of L-dopa and extractive procedure followed by UV assay for its determination in formulations (The United State Pharmacopoeia, 1987). Takashi *et al.,* 2011 tried different solvent ratios of Acetonitrile, formic acid and water for the extraction of L-DOPA from *M. pruriens* seed powder. Mishra and Wagner, 2007, defatted the seed powder with acetone and subjected to water: ethanol (1:1) with 0.1 % ascorbic acid for 3 times by shaking overnight. Vachhani *et al.,* 2011 suggested extraction by cold maceration technique in Water: Methanol mix (50:50) (v/v). Heat reflux in 0.1N HCl of seed powder was suggested by Raina and Khatri, 2011. Kasture *et al.,* 2014 treated with water: ethanol 30:70 (v/v), kept in tightly closed container for seven days. HPLC followed by soxhlet extraction of seed powder was performed by Singh *et al.,* 2010. A quantitative estimation of L-dopa in tablets has been reported by high performance thin layer chromatography method.

Material and Methods

Collection and preparation of Sample

Mucuna pruriens pods were collected from Sanjay Gandhi National Park, Borivali; Mumbai, India on February 2015. 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 by Pallav Chemicals. 1000 ppm standard was prepared by dissolving 100 mg in 10 mL 0.1 N HCl and diluted upto 100 mL by methanol (Rathod and Patel, 2014).

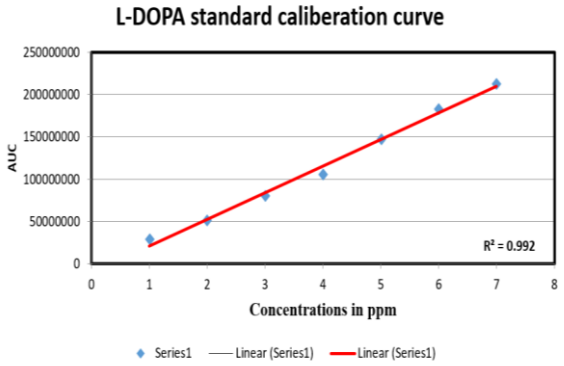
Chromatographic conditions and instrumentation

Chromatographic separation was performed with AGILENT HPLC (Model no. 1220 Infinity) equipped with quaternary pump and auto injector (20µl). Open Lab CDS Version A.04.06 chromatographic software was used for data acquisition. Kromasil 100-5-C18 (250mm × 4.6mm × 5µ); Part/Serial No: M05CLA25/E117509 column was used for analysis. Mobile Phase used was Water/ Methanol/AcetoNitrile (100:60:40) (v/v) containing 0.2% Triethylamine, pH = 3.3 was filtered through 0.45 micron in membrane filter (Millipore) and degassed by sonication; flow rate of 1ml/min was maintained throughout the run. Column effluent was monitored at 280 nm with variable wavelength UV detector (Rathod and Patel, 2014).

Linearity

Working dilutions of L-DOPA in the range of 100–700 ppm was prepared by taking suitable aliquots of working standard solutions (1000 ppm) in different 10ml of volumetric flasks and diluting up to the mark with Methanol was done. 20µl was injected each time into the column at flow rate of 1ml/min. The standard in elute was monitored at 280 nm and corresponding chromatogram were obtained, from these chromatograms peak area were calculated and plot of peak area over concentration was constructed. The correlation coefficient value was found to be 0.992.

Graph 1: Linearity Graph



Preparation of the Plant extracts

Extracts were made using various techniques as listed below:

Method 1:

This method was proposed by Takashi *et al.,* 2011. The preparation of the sample remained the same and only the solvents used for the extraction have been changed so as to check the extraction efficiency of the same procedure with different concentration. The various Solvent systems used were:

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

Method 2 (Misra and Wagner, 2007):

The seed powder was deffated with acetone and then suspended in water: ethanol (1:1) with 0.1 % ascorbic acid for 3 overnights. This was performed with regular change of solvents.

Method 4 (Vachhani, *et al.,* 2011):

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

Method 5 (Raina and Khatri, 2011):

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

Method 6 (Kasture, *et al.,* 2014)

1. The *Mucuna pruriens* powder was treated with water: ethanol 30:70, kept in tightly closed container for 7 days.
2. The supernatant was separated.

Method 7 (Singh, *et al.,* 2010):

1. Seed Powder of the *M. pruriens* was extracted with methanol using a soxhlet apparatus.
2. Extract was dried and was dissolved in 0.1 N HCl.

Results and Discussions

The Standard L-DOPA shows retention time of 2.363 mins as shown in Fig 1.

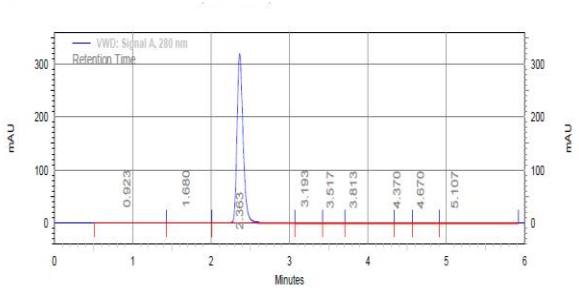


Figure 1: Chromatogram of the Standard L-DOPA

Figure 2 represents the chromatogram of extract made by method 1.3 showing retention time 2.323 mins, confirming the presence of L-DOPA and shows slight tailing, this can be because of high concentration of L-DOPA.

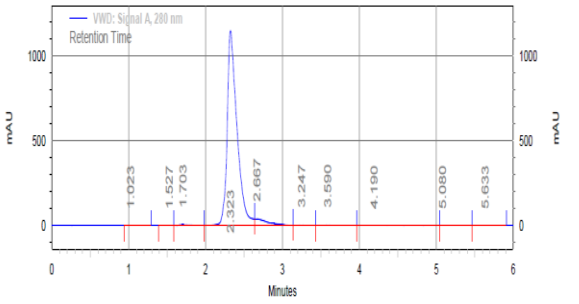


Figure 2: Chromatogram of Plant Extract by Method 1.3

Figure 3 represents plant sample extracted by Method and shows a peak at 2.333 min also indicating the presence of L-DOPA.

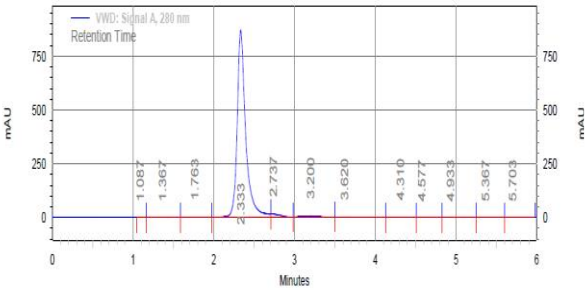


Figure 3: Chromatogram of Plant Extract by Method 2

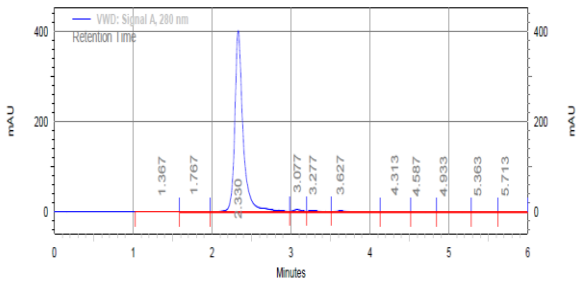


Figure 4: Chromatogram of Plant Extract by Method 4

Plant sample extracted by method 4 shown in Figure 4 also shows a peak at 2.330 mins, indicating the presence of L-DOPA.

Figure 5 indicates the presence of L-DOPA in the plant sample extracted by method 5 as it also shows the retention time of 2.390 min.

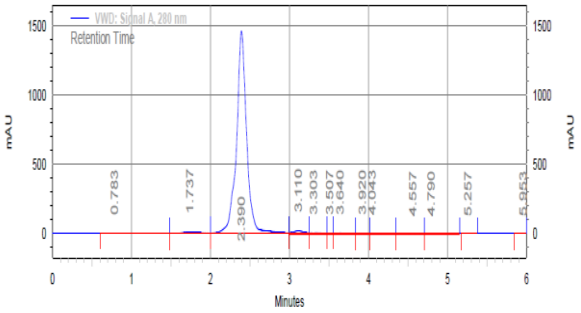


Figure 5: Chromatogram of Plant Extract by Method 5

This confirms, irrespective of the extraction efficiency, there is presence of L-DOPA in the seed powder of *Mucuna pruriens* for quantitation purposes all the plant extract was 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:

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

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

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

Where,

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

The HPLC method discussed in the present work provides a convenient and accurate way for analysis of L-Dopa. 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 1.

Table 1: Comparison of extraction efficiency of various methods

Method of Extraction	Mean Content of L-DOPA extracted (%)
1.1	2.257
1.2	4.157
1.3	4.806
1.4	0.958
1.5	0.843
2	8.810
4	13.362
5	8.722
6	2.134

Method 1 gave a good separation with various subparts of different solvent ratios used, Acetonitrile: Formic acid (100:1), viz., Method 1.3 gave the best extraction efficiency of 4.8% amongst all solvent combination used in Method 1. Method 2 was good method in terms of its extraction efficiency in giving 8.81% L-DOPA in *M. pruriens* but was tedious to perform in comparison to other methods and also was a long procedure. Method 4, a cold maceration technique, gave 13.36% of L-DOPA, which was highest among all the method performed. Method 5 also gave well separated peaks and showed 8.7% extraction efficiency. Method 6 was not as efficient and gave 2.1% of L-DOPA. Method 7, which was the Soxhlet method did not give well separated peak and hence was not considered.

Conclusion

Use of suitable extraction methods will increase the many uses of *Mucuna pruriens* seed with higher levels of active ingredient for the treatment of diseases like Parkinson's. The present investigation suggests cold maceration to remain the best technique from the methods performed for maximum extraction of L-DOPA from all the *Mucuna pruriens* seed powder. Method 4 described with a little modification gives the highest yield of L-DOPA. 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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