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HPLC Estimation of L-DOPA in *Phaseolus vulgaris* and *Cyamopsis tetragonoloba* for the Anti-parkinson's Activity

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Abstract

Aim: The present study aims at finding the presence of Levodopa (L-DOPA) in pods of two widely recognized beans of Leguminosae family namely *Phaseolus vulgaris* and *Cyamopsis tetragonoloba*. L-DOPA (L- 3,4 – dihydroxyphenylalanine) is used as a gold standard to ease the symptoms of Parkinson's disease. The disease is associated with gradual loss of nerve cells in the brain and decreased the ability to synthesize dopamine, causing motor symptoms. *Phaseolus vulgaris* also called common bean consumed enormously throughout the world and is the main source of dietary protein in America and African countries. *Cyamopsis tetragonoloba* (Guar) is a legume crop found in an arid region of India and Pakistan, India is the 90% contributor of total Guar bean production. Both the plants possess nutritive value due to the presence of a high amount of Amino acids and protein content. HPLC technique is applied for the quantitative estimation of L-DOPA present in pods of both the plants. **Methods:** RP-C18 column was used with a mobile phase composed of methanol: water (20: 80), isocratically eluted with a flow rate of 1mL/min. Sample quantity injected was 20 µL. The UV detection wavelength was 280 nm and retention time noted as 7.269±0.3min. Calibration curve obtained by injecting different concentration range of standard solution gives regression equation as Y= 10.154x + 1.4696 and correlation coefficient as 0.998. The amount of L-DOPA present in both the plants were measured with respect to standard levodopa. **Result and Conclusion:** The amount of levodopa present in *Phaseolus vulgaris* was found to be 0.566% and 0.174% in *Cyamopsis tetragonoloba*, which shows that both the plants contain L-DOPA in their pods. It can be concluded that RP-HPLC technique can be applied for quantitative estimation of levodopa present in plant extract and both the plants can be explored further to use in herbal drug formulations and can provide an alternative source of L-DOPA from a natural source to ease symptoms of Parkinson's disease.

Keywords
Cyamopsis tetragonoloba, Levodopa, Natural sources, *Phaseolus vulgaris*, Parkinson's disease.

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INTRODUCTION

L-DOPA (L- 3, 4 – dihydroxyphenylalanine) is a precursor for the neurotransmitter dopamine. It is essential and formed in the human body, the absence of L-DOPA (Levodopa) Figure 1,2 [8] can cause many chronic diseases including a neurodegenerative disease called Parkinson's disease. Parkinson's disease is recognized by impairment of motor and non-motor functions. Dopamine cannot be given directly as it is unable to cross the blood-brain barrier. L-DOPA is capable of crossing a blood-brain barrier and get converted to dopamine, which is metabolized by catechol-o-methyl transferase (COMT). For Parkinson's treatment L-DOPA is considered as the gold standard [1]. It was first isolated from *Vicia faba* (legume) in 1910 [2]. Legumes have been considered a good source of bioactive components and are proven to be useful in many chronic degenerative diseases and are effective in reducing oxidative stress [3]. *Phaseolus vulgaris* (family Fabaceae) also known as common bean, is a tropical American crop also cultivates in the regions of India and Pakistan [4]. *Phaeolus vulgaris* possess various amino acids like phenylalanine and tyrosine in its root and leaves. Dopamine is a tyrosine derivative. Tyrosine is converted further by tyrosine hydroxylase to L-DOPA which with the help of DOPA decarboxylase converts to dopamine. *Phaseolus vulgaris* is also considered a good source to treat nervous irritability [5]. *Cyamopsis tetragonoloba* is an annual legume grown in India and Pakistan and also known as guar, it possesses many anti-estrogenic, anti-histamine, anti-diabetic activities. Studies performed on guar revealed its good radical scavenging abilities [6]. Amino acid profiling of guar also showed a very high amount of Lysine and Arginine and relatively sufficient amount of tyrosine. Guar is a rich source of crude protein after soya bean meal due to its bulk Argintine content [7]. *Mucuna pruriens* (Leguminosae) is very widely used for the extraction of L-DOPA and consider one of the best source [8], So the present study is focused on finding a potential source of L-DOPA in *Phaseolus vulgaris* and *Cyamopsis tetragonoloba* for Anti-Parkinson's activity with the help of HPLC technique.

MATERIALS AND METHODS

Reagent and Material

The standard Levodopa L-DOPA was provided by Scan Research laboratory, Indrapuri, Bhopal. Methanol, water and Acetonitrile have been of HPLC grade purchased from Merck Ltd, New Delhi, India. All the other chemicals and solvent used were of HPLC and analytical grade. Plants *Cyamopsis tetragonoloba* and *Phaseolus vulgaris* (Pods) were Identified and Authenticated by Dr. Zia ul Hasan, Head of department, faculty of Science, Saifia College, Bhopal, (M.P).

Instrumentation

Absorption measurements was performed on Double beam UV-Visible Spectrophotometer (a Thermospectronic version of Labindia 3000) Model - 108 with 1cm matched quartz cells. Spectral range was 200-400 nm. The HPLC (waters) consist of a pump, UV Detector, a Thermo C18 Octadecylsilane (250X4.6 mm. 5 µm) column, a Data Ace software program.

Chromatographic Condition

HPLC evaluation was performed under ambient chromatographic temperature on RP-C18 analytical column. Mobile phase used were Methanol: Water (20: 80 v/v) with a Flow rate of 1 mL/min isocratically eluted. A sample quantity of 20µL was injected into a sample injector. Washing of column was done by eluting mobile phase for 30 min and run the baseline. The chromatogram was monitored with UV detection wavelength of 280 nm. Retention time was found to be 7.269± 0.3 min.

Preparation of Standard Stock Solution

A standard stock solution of 1000 µg/ml was prepared by dissolving accurately weighed 10 mg of L-DOPA and was transferred to a 10ml volumetric flask and the volume is adjusted by adding methanol.

Working Standard solution

A working standard solution was prepared by diluting the standard stock solution of L-DOPA. 0.05, 0.1, 0.15, 0.20 and 0.25 ml was taken and diluted up to 10 ml by adding methanol. Final concentration range prepared was 5, 10, 15, 20, 25 µg/ml.

Sample Preparation

10 mg of methanol extract of *Cyamopsis tetragonoloba* and *Phaseolus vulgaris* (Pods) were accurately weighed and volume was adjusted to 10 ml with methanol to make the resultant sample solution of 1000 µg/ml. The solution was filtered through 0.45 µm membrane filter and further used for HPLC analysis.

RESULTS

Linearity and Range

The HPLC evaluation is carried out in RP-C18 analytical column. A calibration curve is prepared by injecting standard solution of concentration range 5, 10, 15, 20, 25 µg/ml. Linearity was tested by calculating average peak area of L-DOPA of different injection volume. The curve obtained between Peak Area and different concentration of standard gives

the regression equation and correlation coefficient of curve. Shown in order as Table 1, Figure 3.
Further Characteristics and specification of Standard curve is given in Table 2. The Chromatograms of standard and Extracts of *Phaseolus vulgaris* and *Cyamopsis tetragonoloba* are shown in figure 4,5,6 and the final percentage yield and Area of standard along with plant extracts are shown under Table 3.

Table 1. Linearity and Range of standard L-DOPA

S.N	Concentration of L-DOPA	Peak Area
1.	0	0
2.	5	48.768
3.	10	107.673
4.	15	155.369
5.	20	208.569
6.	25	249.954

Table 2: Characteristics of Standard Calibration Curve

Compound	Linearity µg/ml	Correlation Coefficient	Slope	Intercept
Leavodopa (L-DOPA)	5-25	0.9982	10.154	1.469

Table 3: **Amount of Levodopa (in %) in *Cyamopsis tetragonoloba* and *Phaseolus vulgaris***

Extract	Peak Area	% Yield
Levodopa	155.162	1.516
Phaseolus vulgaris	58.978	0.566
Cyamopsis tetragonoloba	19.160	0.174

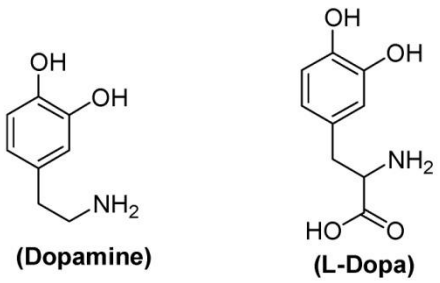

Figure :1 Structure of Dopamine, L-DOPA [8]

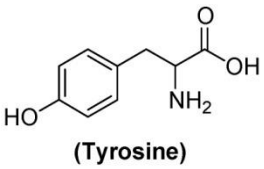

Figure 2: Tyrosine [8]

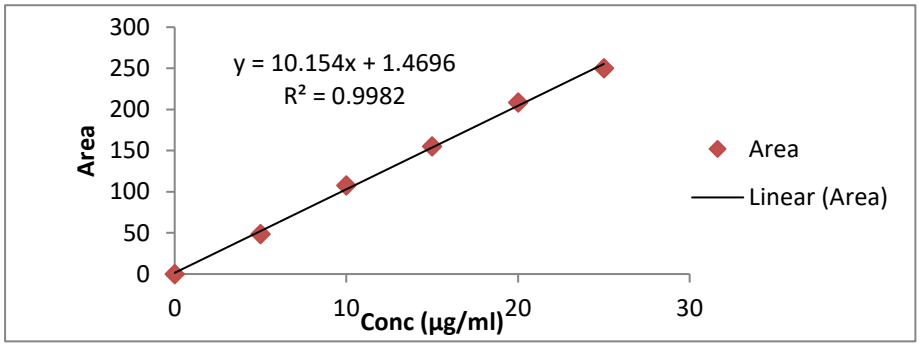

Figure 3: Calibration curve of L-DOPA

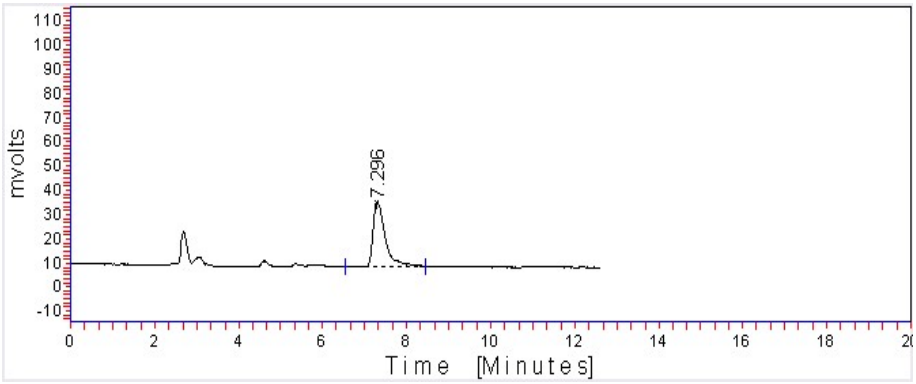

Figure 4: Standard HPLC Chromatogram of Levodopa (L-DOPA)

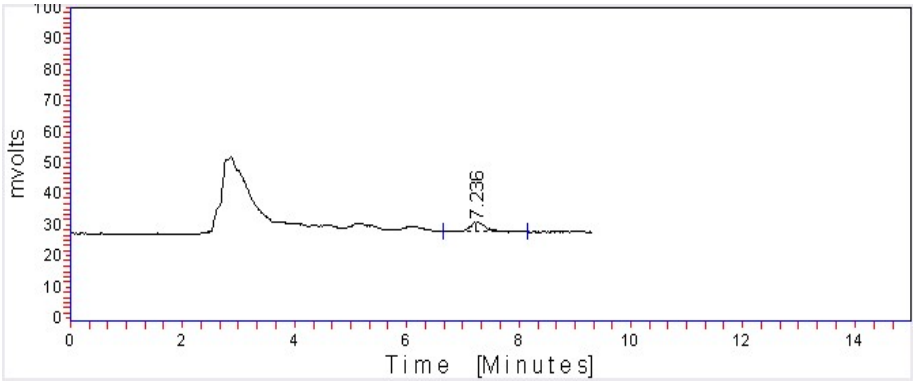

Figure 5: HPLC Chromatogram of *Phaseolus vulgaris*

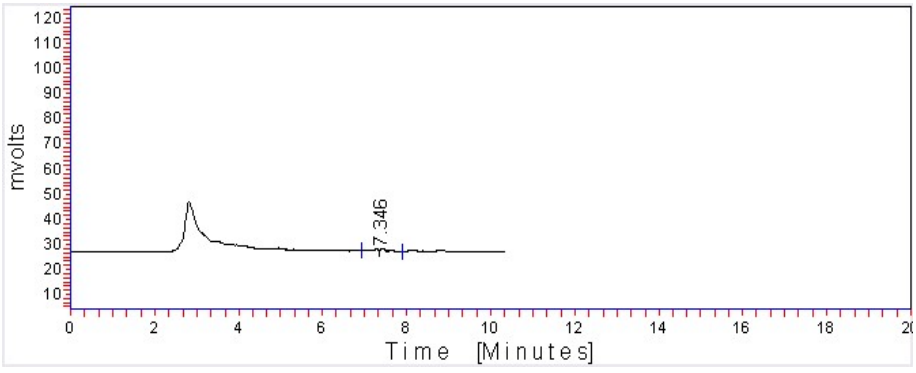

Figure 6: HPLC Chromatogram of *Cyamopsis tetragonoloba*

DISCUSSION

HPLC is conducted under appropriate chromatographic conditions, stationary phase was selected depending upon the sample extract. Mobile phase methanol: water (20: 80 v/v) was selected and its composition effect on retention time of levodopa was carefully noted. The calibration curve for L-DOPA was prepared with concentration verses area under the peak that gives the line of regression equation as Y= 10.154x + 1.4696 and correlation coefficient as 0.9982. On the analysis it was found that both *Cyamopsis tetragonoloba* and *Phaseolus vulgaris* confirm the presence of Levodopa in their pods. The amount of levodopa present in *Phaseolus vulgaris* was found to be 0.566% and *Cyamopsis tetragonoloba* showed the amount as 0.174%. The results clearly indicate that *Phaseolus vulgaris* shows comparatively higher amount of levodopa than *Cyamopsis tetragonoloba* pods. Which can be used as a potent source to ease the symptoms of Parkinson's disease. Earlier it was established that *Phaseolus vulgaris* possess reasonable amount of L-DOPA in its leaves and roots. The present study successfully confirms the presence of levodopa in its pods as well. On the other hand, *Cyamopsis tetragonoloba* have shown the presence of Tyrosine (an L-DOPA precursor) in its leaves and roots. HPLC analysis reveals relatively smaller amount of levodopa present in plant pod of guar, but the whole plant can be used for its Anti-Parkinson's activity for the presence of both Tyrosine and L-DOPA.

CONCLUSION

By looking at the above analysis it is confirmed that both the plants possess levodopa in their pods, which is conclusive of its use as potent drug for Anti-Parkinson's activity. The plant belongs to Leguminosae family have higher probability of finding Levodopa as proven by earlier studies. On that basis the studies were conducted and obtained positive results. The plants can be used as therapeutics and in herbal formulations of L-DOPA. The plants are readily available in large amount during monsoon season and possess good medicinal value. Using plant extract can be very cost effective and cheap and causes less side effect in terms of synthetic drug available commercially. The current investigation can be resourceful for understanding alternative sources for preparing formulations for Parkinson's disease.

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