International Journal of Botany

ISSN: 1811-9700

OPEN ACCESS International Journal of Botany

ISSN 1811-9700
DOI: 10.3923/ijb.2025.21.27

Research Article

Pharmacognostical Analysis and Quantification of L-Dopa Content in *Mucuna pruriens* (L.) DC

[1]Vinod Kumar Bisht, [2]Ramesh Chandra Uniyal, [1]Santosh Baliram Dhutraj, [3]Partha Ganguly, [3]Rahul Singh and [4]Rajiva Kumar Rai

[1]Zandu Foundation for Health Care, Ambach, Valsad, Gujarat 396145, India
[2]Department of Bioresources, Emami Limited, 687 Anandapur, EM Bypass, Kolkata, West Bengal 700107, India
[3]Corporate Analytical Design Excellence, Emami Limited, 13, BT Road, Belghoria, Kolkata 700056, India
[4]R&D Healthcare Division, Emami Limited, 13, BT Road, Belghoria, Kolkata, West Bengal 700056, India

Abstract

Background and Objective: *Mucuna pruriens* commonly known as velvet bean is well known for its L-Dopa content. The pods of the *M. pruriens* are prone to insect attacks, which reduces the yield of seeds. This study aimed to optimize the best time for pod harvesting to ensure higher L-Dopa content and improved seed quality. **Materials and Methods:** Seeds of *M. pruriens* were collected at different stages of pod growth and maturation viz. S_1: At initial stage of pod formation, S_2: At 15 days after pod formation, S_3: At 30 days after pod formation, S_4: At 45 days after pod formation and S_5: Mature pod at 60 days. These seeds were analyzed for total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive, fixed oil content and L-Dopa content. **Results:** The study revealed that total ash content was lowest in S_1 followed by S_3. The acid insoluble ash content was found a minimum of 0.13% in S_3 and a maximum of 0.44% in S_5. The alcohol soluble extractive and water-soluble extractive were found highest in S_3 with the value of 6.47 and 24.99%, respectively. Fixed oil analysis also shows the presence of the highest percent of fixed oil in S_4 (3.84%) followed by S_3 with 3.79%. The HPLC analysis of L-Dopa in seed samples of *M. pruriens* revealed the presence of the highest content of 6.08% in S_5 followed by 6.01% in S_3. **Conclusion:** Overall results revealed that S_3 is the best time for pod harvesting in *M. pruriens*.

Key words: Total ash content, acid insoluble ash, alcohol soluble extractive, water soluble extractive, high performance liquid chromatography

Citation: Bisht, V.K., R.C. Uniyal, S.B. Dhutraj, P. Ganguly, R. Singh and R.K. Rai 2025. Pharmacognostical analysis and quantification of L-Dopa content in *Mucuna pruriens* (L.) DC. Int. J. Bot., 21: 21-27.

Corresponding Author: Vinod Kumar Bisht, Zandu Foundation for Health Care, Ambach, Valsad, Gujarat 396145, India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The velvet bean, *Mucuna pruriens* (L.) DC., belongs to the family Fabaceae is an annual twinning climber, grown worldwide as food, feed, fodder and cover crop[1]. Its seeds are considered highly nutritional and possess valuable medicinal properties[2]. Seeds of *M. pruriens* are used against a wide range of diseases including neurological disorders, urinary tract infections, menstruation disorders, edema, fever, tuberculosis, constipation, ulcers and Parkinson's diseases[3-5]. In addition, *M. pruriens* possess anti-diabetic, anti-neoplastic, anti-epileptic, antimicrobial, antioxidant, anti-inflammatory and anthelmintic activities[6,7]. In India, the tonic prepared from the seeds of *M. pruriens* is used for male virility[8]. The seeds of *M. pruriens* are used as a constituent in more than 200 indigenous drug formulations[4].

The plant is cultivated primarily for its seeds, which are the best-known natural source of the aromatic amino acid, L-3,4-dihydroxy phenylalanine (Levodopa or L-Dopa). The L-Dopa is a direct precursor to the neurotransmitter dopamine, that serves as the gold standard for the treatment of Parkinsonism[1]. *Mucuna pruriens* seeds are used for the preparation of Ayurvedic medicine for the treatment of Parkinson's disease[7,9]. The L-Dopa was first isolated from the seeds of *M. pruriens* in 1937 and it is reported that the concentration ranged between 4-6%[2]. After the discovery that L-Dopa contains anti-parkinson activity, the demand for L-Dopa in national and international markets has increased sharply[7]. Higher anti-Parkinsonian activity was reported for the natural L-Dopa available in seed powder as compared to synthetic L-Dopa[10,11].

The Ayurvedic industry follows the parameters of Ayurvedic Pharmacopoeia of India (API) for the preparation of drugs from *M. pruriens*, thus not only the L-Dopa content but the *M. pruriens* raw material should also need to comply with API parameters. Therefore, the present study was carried out to analyze the seeds of *M. pruriens* for pharmacognostical and L-Dopa content.

MATERIALS AND METHODS

Study area: The study was conducted from June, 2020 to February, 2021 at the research station of Zandu Foundation for Health Care, Ambach, Valsad, Gujarat (20.40°N Latitude and 72.98°E Longitude; Altitude: 15 M.A.S.L.).

Plant material and reagents: Seeds of non-itching line (ZFA 112) developed by the Zandu Foundation for Health Care through conventional breeding[12] were used for the present study. The seed germination took place within 2 weeks of sowing. Pod formation starts 120 days after seed germination. The pods were collected at different stages of plant growth right from pod formation to pod maturation, from three replicates of 50 plants each and pooled together to avoid any bias. The pods collection stages were abbreviated as S_1 to S_5 as, S_1: At the initial stage of pod formation, S_2: At 15 days after pod formation, S_3: At 30 days after pod formation, S_4: At 45 days after pod formation and S_5: At 60 days after pod formation. Each pod possessed 3-6 seeds. The seeds were extracted from the pods and grinded through pulverizer and particles passed through a 0.35 mm sieve were taken for pharmacognostical analysis and L-Dopa content analysis.

Reagents and standard: Standard L-Dopa were procured from Loba Chemie, Mumbai, India and chemicals used for L-Dopa analysis were of HPLC grade, obtained from Merck, Mumbai (India), while for pharmacognostical analysis, analytical grade chemicals obtained from Himedia (India) were used.

Preparation of samples and pharmacognostical analysis: Total ash content, acid insoluble ash content, alcohol soluble extractive, and water-soluble extractive were analyzed following the procedures outlined in the Ayurvedic Pharmacopoeia of India. Fixed oil content analysis (esters of higher fatty acids and glycerin) was also carried out.

Extraction and preparation of sample solutions for HPLC: For chromatographic analysis, accurately weighed 500 mg powdered seeds from each sample were extracted with 50 mL of 0.01 (M) HCl using sonication for 15 min. The resulting solution was filtered through Whatman No. 1 filter paper into a100 mL volumetric flask and the volume was made up of 0.01 (M) HCl, then filtered through a 0.20 µm syringe filter and used for HPLC analysis. Accurately 10 µL of each sample (5 mg/mL) was injected into the HPLC system individually.

Preparation of standard solutions for HPLC: An accurately weighed 50 mg of L-Dopa was dissolved in 50 mL of 0.01 (M) HCl to get 1 mg/mL stock solution. Working standards of 50, 100, 200, 300 and 500 µg/mL were prepared separately by serial dilution of the stock solutions. The calibration curve for the standard was linear, with a correlation coefficient of 1.00.

HPLC conditions: For the HPLC study, the Shimadzu (Japan) made LC-2010C_{HT} quaternary HPLC equipped with auto-injector and UV-VIS detector was used for the present study. Integration was carried out using the LC Solutions software (version 1.25) while Phenomenex made Gemini C_{18} column (250×4.6 mm, 5 µ ID) was used for separation. The solvent used for the study was comprised of 1.36 g of potassium dihydrogen phosphate as a buffer in the 1000 mL water. The pH of the solution was adjusted to 3.5 by orthophosphoric acid and methanol (10:90). The flow rate was maintained at 0.5 mL/min to elute the analyte isocratically and detected at 282 nm by UV-VIS detector having a range of 200-400 nm. The column was kept at ambient conditions. The exact 10 µL volume of the standard and the samples were injected in triplicate and the average peak area was recorded and analyzed for quantification.

System suitability test: Before proceeding with the sample analysis, equilibrate the column until a stable baseline is obtained. Check that the percentage of RSD among the three injections of the standard solution must not be more than 2.0%. If the above criteria are passed, proceed with the sample analysis or else make adjustments to meet the system suitability parameters.

RESULTS AND DISCUSSION

The results of the pharmacognostical and chemical analysis are presented in Table 1. Against the limit of not more than 5% in API, the total ash content was found within the range for all the samples studied, however, was observed minimum in S_1. Ash contains inorganic materials such as carbonates and silicates of sodium, potassium, magnesium, calcium, etc. and is used to determine the quality and purity of the raw drug. Likewise, all the samples comply with the permissible limit for acid insoluble ash content specified in API. The alcohol soluble and water-soluble extractive was found highest in S_3 with the value of 6.47 and 24.99%, respectively, however, other samples also complied with the API standards (Table 1). Fixed oil content in all the samples was within the API limits for all the samples, with the highest value observed in S4 (3.84%), followed by S3 (3.79%). Thus, the values of total ash content, acid insoluble ash content, alcohol soluble extractive, water soluble extractive and fixed oil content in *M. pruriens* seeds were well within the acceptable range for all the samples studied as per API standards.

The HPLC analysis revealed comparable variations in the L-Dopa among the seed samples of *M. pruriens* (Fig. 1-7). The HPLC analysis of standard L-Dopa showed their average retention time (t_R) at 5.40 min with the correlation coefficient (R^2) of 1.00. In HPLC analysis, the highest content of L-Dopa (6.08%) was found in the seeds harvested at 60 days after pod formation (S_5) followed by S_3 (6.01%), S_1 (5.99%) and S_2 (5.85%). Least concentration of 5.19% of L-Dopa was found in sample S_4 (Table 1).

Table 1: Pharmacognostical and chemical analysis of seeds of *M. pruriens* at different stages of pods growth

		Stages of pods growth[b]				
Parameters (% w/w)	API standard[a]	S_1	S_2	S_3	S_4	S_5
Total ash	NMT[C] 5%	3.42	3.56	3.55	3.76	3.67
Acid insoluble ash	NMT 1%	0.29	0.25	0.13	0.34	0.44
Alcohol soluble extractive	NLT[d] 3%	5.27	6.36	6.47	5.52	4.93
Water soluble extractive	NLT 23%	23.88	24.06	24.99	23.18	23.86
Fixed oil content	NLT 3%	3.32	3.12	3.79	3.84	3.21
L-Dopa content	-	5.99	5.85	6.01	5.19	6.08

[a]API: Ayurvedic Pharmacopoeia of India, [b]S_1: Initial stage of pod formation, S_2: At 15 days after pod formation, S_3: At 30 days after pod formation, S_4: At 45 days after pod formation and S_5: Mature pod at 60 days, [c]NMT: Not more than and [d]NLT: Not less than

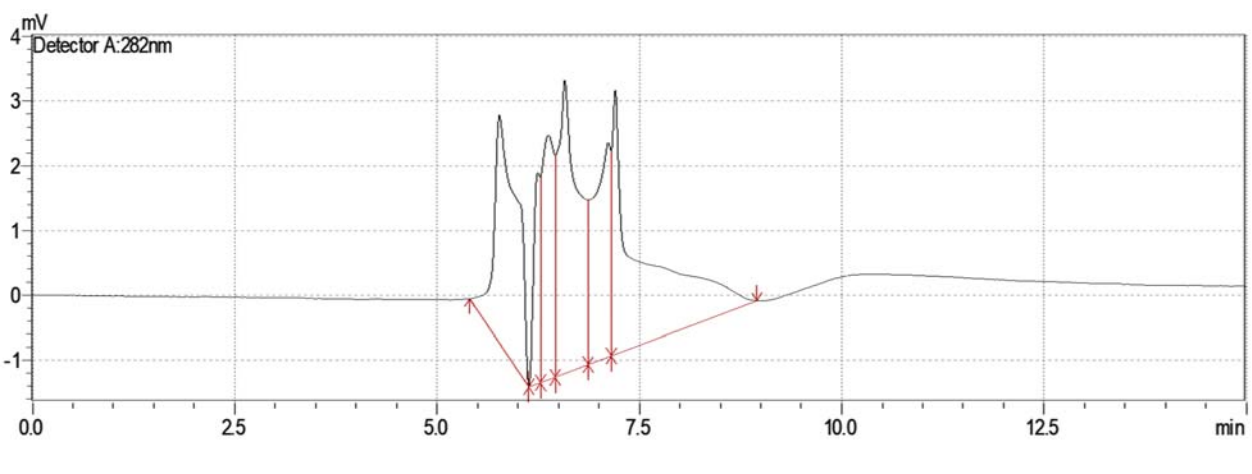

Fig. 1: HPLC chromatogram of diluent (Blank)

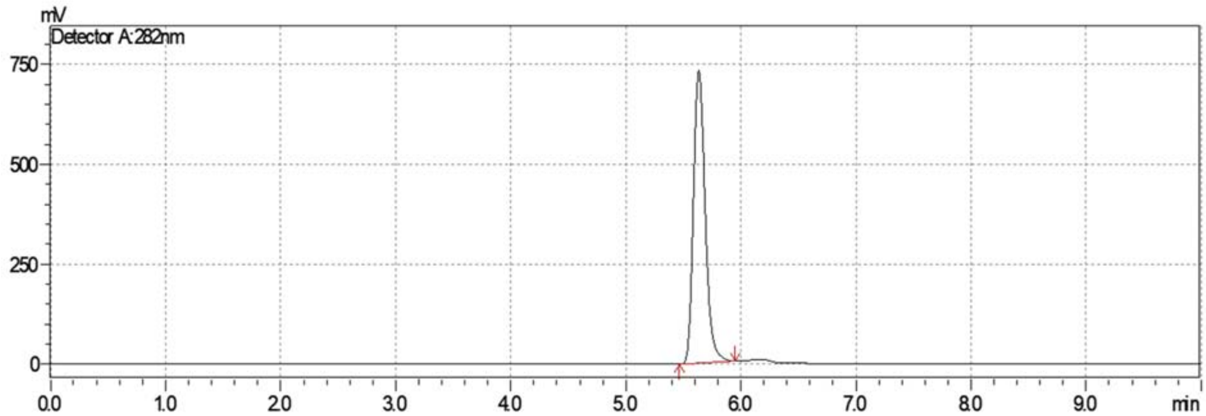

Fig. 2: HPLC chromatogram of L-Dopa standard

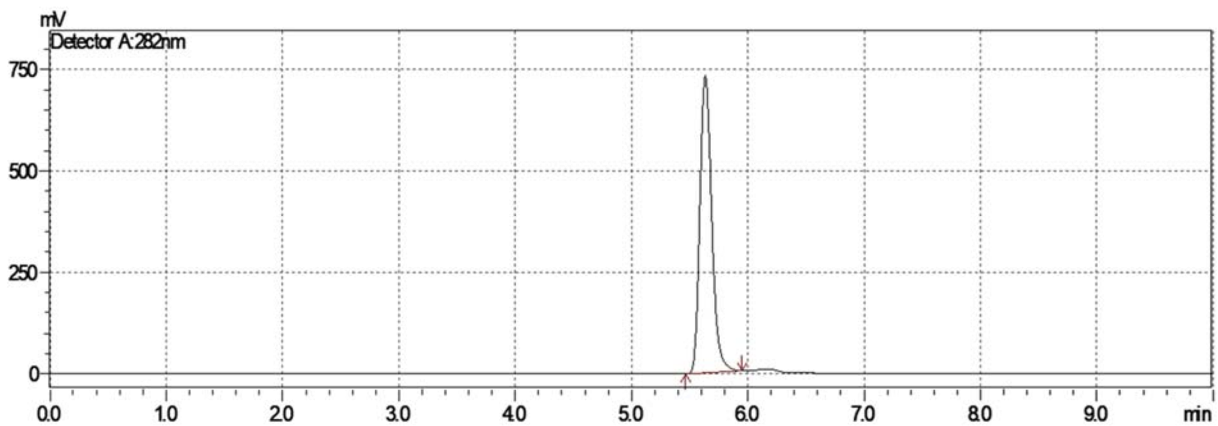

Fig. 3: HPLC chromatogram of L-Dopa in S_1

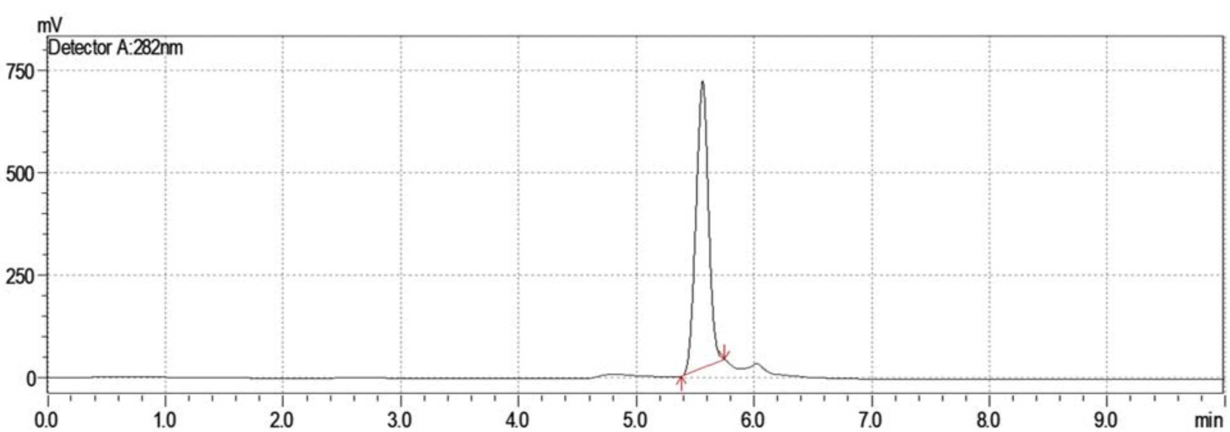

Fig. 4: HPLC chromatogram of L-Dopa in S_2

In the present study, *M. pruriens* seeds contain comparatively high concentrations of L-Dopa (5.19-6.08%). Previous studies have reported the concentration of L-Dopa ranging between 2.94-6.91[13], 3.9-10.60[14], 2.23-5.36[15], 3.1-6.1[4] and 4-6%[8].

The presence of a comparably high concentration (5.99%) of L-Dopa in the initial stage of pod formation, as observed in the present study is an interesting finding. This indicated that L-Dopa might be synthesized in leaves and later on transported to the pods right from its formation. Synthesis of

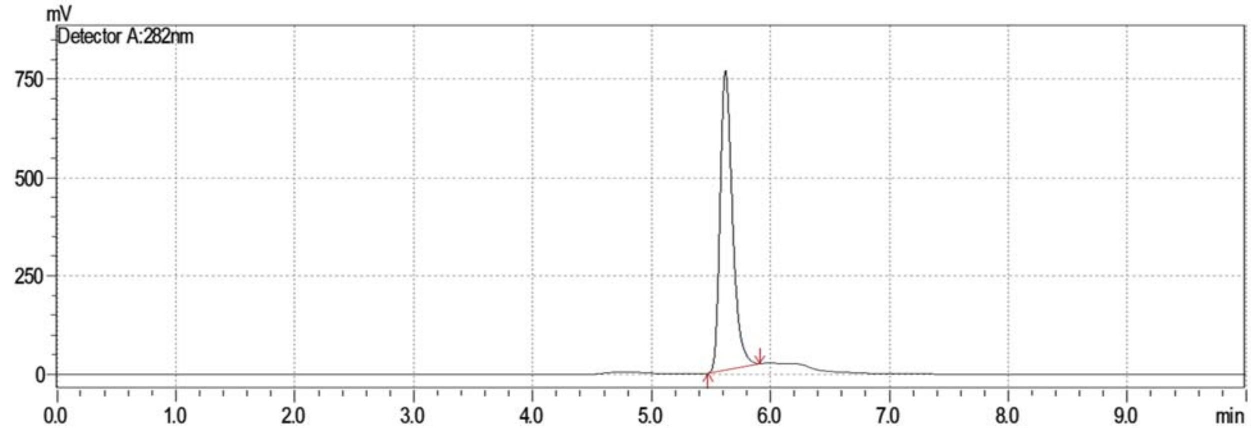

Fig. 5: HPLC chromatogram of L-Dopa in S_3

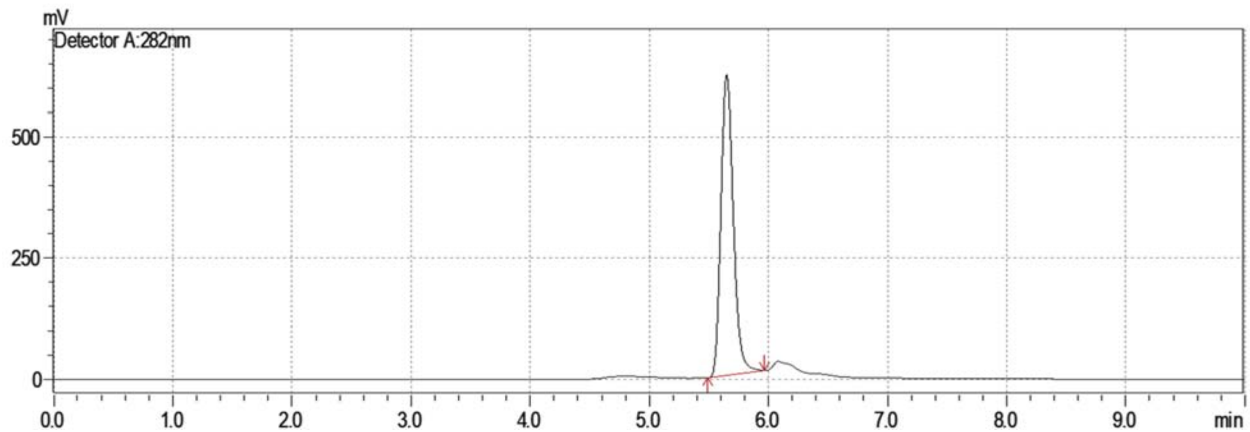

Fig. 6: HPLC chromatogram of L-Dopa in S_4

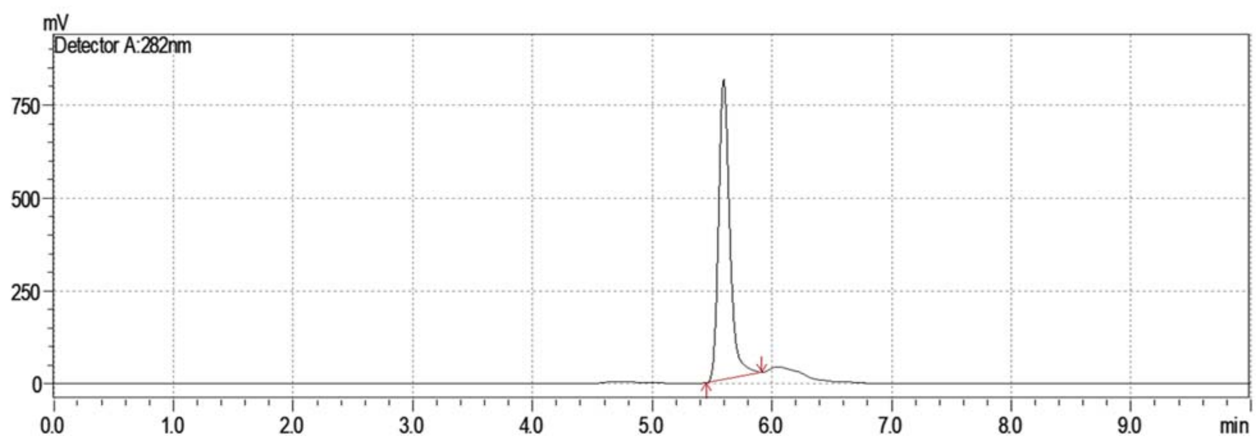

Fig. 7: HPLC chromatogram of L-Dopa in S_5

active ingredients in leaves and their storage in pods are adaptive characteristics in many plant species[16]. Another study has shown that the highest concentration of L-Dopa content was detected in leaves at physiologically mature plants of *Vicia faba*[17]. If this assumption hold true, then leaves could potentially serve as an alternative source of L-Dopa instead of seeds. However, further investigations for the quantification of L-Dopa content in leaves are required because it is reported that the leaves contain only about 0.5 to 1.0% L-Dopa[18].

The pods are highly prone to insect attacks, particularly in the latter half of their growth. To check the insect attacks, farmers often resort to using chemical insecticides, which can be absorbed by the pods and accumulate in the seeds. Furthermore, to reduce the progression of Parkinson's disease, long-term use with a gradual increase in the dose of *M. pruriens* is recommended[19] which may exacerbate the risk of severe side effects such as, psychosis, nausea, emesis, arrhythmia and hypertension[20]. Thus, frequent and long-term use of *M. pruriens* seeds having chemical insecticides may significantly increase the risk of inception of several diseases including lethal diseases manifolds. Thus, there is a need to explore some alternate ways to reduce the use of chemical insecticides. One such approach is to find out the optimal time for pod harvesting. Pharmacognostical and L-Dopa analysis of *M. pruriens* conducted in the present study indicated that harvesting of pods can be done after the pods become semi-mature i.e. after S_3 stage. Raina and Khatri[15] reported that better quality seeds (in appearance, shape and colour) with higher yield and optimum L-Dopa content (4.68%) were produced at semi-mature stage. Thus, harvesting of pods at S_3 stage is recommended to get quality raw material, it will also reduce the damages caused due to insect attacks.

CONCLUSION

Pharmacognostical analysis revealed that all the samples studied complied with the parameters of the Ayurvedic Pharmacopoeia of India. However, S_3 stage was found best suited for pod harvesting because of the least acid insoluble content, highest alcohol soluble extractive, water soluble extractive and fixed oil concentration. The total ash and L-Dopa content was second least in all the tested samples. It also reduces the risk of insect attacks as such attacks on *M. pruriens* pods are more severe in the second half of their growth.

SIGNIFICANCE STATEMENT

The significance of the present study lies in its findings which optimize the best time for pod harvesting to ensure higher L-Dopa content and improved seed quality, both of which are critical for therapeutic applications. The results indicate that the best time of pod harvesting is 30 days after pod setting and provide actionable insights that can enhance efficacy of the end product, maintain faith in herbal medicine, improve agricultural practices and contribute to scientific knowledge.

REFERENCES

1. Pulikkalpura, H., R. Kurup, P.J. Mathew and S. Baby, 2015. Levodopa in *Mucuna pruriens* and its degradation. Sci. Rep., Vol. 5. 10.1038/srep11078.
2. Cassani, E., R. Cilia, J. Laguna, M. Barichella and M. Contin *et al*., 2016. *Mucuna pruriens* for Parkinson's disease: Low-cost preparation method, laboratory measures and pharmacokinetics profile. J. Neurol. Sci., 365: 175-180.
3. Katzenschlager, R., A. Evans, A. Manson, P.N. Patsalos and N. Ratnaraj *et al*., 2004. *Mucuna pruriens* in Parkinson's disease: A double blind clinical and pharmacological study. J. Neurol. Neurosurg. Psychiatry, 75: 1672-1677.
4. Kavitha, C. and C. Thangamani, 2014. Amazing bean "*Mucuna pruriens*": A comprehensive review. J. Med. Plants Res., 8: 138-143.
5. Ulu, R., N. Gozel, M. Tuzcu, C. Orhan and İ.P. Yiğit *et al*., 2018. The effects of *Mucuna pruriens* on the renal oxidative stress and transcription factors in high-fructose-fed rats. Food Chem. Toxicol., 118: 526-531.
6. Jalalpure, S.S., K.R. Alagawadi, C.S. Mahajanashetti, B.N. Shah and Salahuddin *et al*., 2007. *In vitro* anthelmintic property of various seed oils against *Pheritima posthuma*. Indian J. Pharm. Sci., 69: 158-160.
7. Lampariello, L.R., A. Cortelazzo, R. Guerranti, C. Sticozzi and G. Valacchi, 2012. The magic velvet bean of *Mucuna pruriens*. J. Tradit. Complementary Med., 2: 331-339.
8. Rai, S.N., H. Birla, W. Zahra, S.S. Singh and S.P. Singh, 2017. Immunomodulation of Parkinson's disease using *Mucuna pruriens* (Mp). J. Chem. Neuroanat., 85: 27-35.
9. Ovallath, S. and P. Deepa, 2013. The history of parkinsonism: Descriptions in ancient Indian medical literature. Mov. Disord., 28: 566-568.
10. Manyam, B.V., M. Dhanasekaran and T.A. Hare, 2004. Effect of antiparkinson drug HP-200 (*Mucuna pruriens*) on the central monoaminergic neurotransmitters. Phytother. Res., 18: 97-101.
11. Manyam, B.V., M. Dhanasekaran and T.A. Hare, 2004. Neuroprotective effects of the antiparkinson drug *Mucuna pruriens*. Phytother. Res., 18: 706-712.
12. Bisht, V.K., J.M. Pathak and R.C. Uniyal, 2020. Zandu Foundation for Health Care (ZFHC): 30 Years of Journey. Academic Publishers, Kolkata, India, ISBN: 9789387162570, Pages: 248.
13. Chinapolaiah, A., K.H. Bindu, G.N. Manjesh, V. Thondaiman, V.K. Rao, N.H. Rao and S.S. Kumar, 2019. Variability in L-Dopa and other biochemical composition of *Mucuna pruriens* (L.) an underutilized tropical legume. Ind. Crops Prod., Vol. 138. 10.1016/j.indcrop.2019.06.010.
14. Yang, X., X. Zhang and R. Zhou, 2001. Determination of L-Dopa content and other significant nitrogenous compounds in the seeds of seven *Mucuna* and *Stizolobium* species in China. Pharm. Biol., 39: 312-316.

15. Raina, A.P. and R. Khatri, 2011. Quantitative determination of L-DOPA in seeds of *Mucuna pruriens* germplasm by high performance thin layer chromatography. Indian J. Pharm. Sci., 73: 459-462.
16. Çiçek, S.S., C.M. Cardenas and U. Girreser, 2022. Determination of total sennosides and sennosides A, B, and A_1in senna leaflets, pods, and tablets by two-dimensional qNMR. Molecules, Vol. 27. 10.3390/molecules27217349.
17. Etemadi, F., M. Hashemi, R. Randhir, O. ZandVakili and A. Ebadi, 2018. Accumulation of L-DOPA in various organs of faba bean and influence of drought, nitrogen stress, and processing methods on L-DOPA yield. Crop J., 6: 426-434.
18. Fujii, Y., T. Shibuya and T. Yasuda, 1992. Allelopathy of velvetbean: Its discrimination and indentification of L-DOPA as a candidate of allelopathic substances. Jpn. Agric. Res. Q., 25: 238-247.
19. Patil, S.A., O.A. Apine, S.N. Surwase, J.P. Jadhav, 2013. Biological sources of L-DOPA: An alternative approach. Adv. Parkinson's Dis., 2: 81-87.
20. Manini, P., M. d'Ischi and G. Prota, 2001. An unusual decarboxylative maillard reaction between L-DOPA and D-glucose under biomimetic conditions: Factors governing competition with pictet-spengler condensation. J. Org. Chem., 66: 5048-5053.