

**Research Article****A COMPARATIVE ASSESSMENT OF PHARMACOLOGICALLY ACTIVE PRINCIPLES AND ANTIOXIDANT ACTIVITY OF COMMONLY OCCURRING *MUCUNA* SPS. IN INDIA****Murthy S N^{1*}, Manasi M. Malgaonkar², A. R. Shirolkar³, S. D.Pawar⁴, S. Sangvikar⁴, Y.R.Kulkarni⁵**

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ABSTRACT

The genus *Mucuna* (Fabaceae) includes about 150 species and almost all the species are reported to contain L-3,4-dihydroxy phenylalanine (L-Dopa), a non-protein amino acid that acts as precursor for the neurotransmitter dopamine, used in the treatment of Parkinson's disease. The present study was aimed to assess the pharmacologically active principles and antioxidant activities of *Mucuna pruriens* (L.) DC., *Mucuna bracteata* DC., *Mucuna cochinchinensis* (Lour.) A. Chev. black seeds sp. and *Mucuna cochinchinensis* (Lour.) A. Chev. white seeds sp. The aqueous extract of plant material was subjected to assess the pharmacologically active principles by using L-dopa quantification, determination of protein and flavonoids, total phenolic content, Inhibition of DPPH radical and Reducing power/Ferric reducing antioxidant potential (FRAP) assays. The results obtained showed that, *Mucuna cochinchinensis* black seeds sp. has highest protein, total phenolic content, L-dopa content and DPPH radical scavenging activity. *Mucuna bracteata* seeds showed highest flavonoid content whereas, in FRAP assay *Mucuna pruriens* extract showed highest potential to reduce the ferric ions. It can be concluded from this study that, L-DOPA possesses antioxidant activity which was supported by *in-vitro* antioxidant assays. This indicates that the antioxidant activity of the aqueous extracts of all the *Mucuna* sps. may be due to the presence of L-Dopa content in its seed.

KEYWORDS: *Mucuna* sps., L-dopa, Antioxidants activity, flavonoids etc.

INTRODUCTION

The genus *Mucuna* belongs to the family Fabaceae (Leguminosae) and includes about 150 species of annual and perennial legumes of pan-tropical distribution. India is one of the natural centers of origin of the *Mucuna* in the world^[1]. To date fifteen species of *Mucuna* species are reported from India. Many species of the genus offer an excellent source as cover crop and green manure, in addition to their traditional use as feed and food^[2,3]. Almost all the species are reported to be used in the treatment of Parkinson's disease^[4]. Kapikacchu (*Mucuna*) is an ingredient of several commercial preparations that are being used as an aphrodisiac, in male sexual disorders. Seeds are astringent, laxative, anthelmintic, alexipharmic, hypotensive, spasmodic, hypocholesterolemic, antifungal, anti-inflammatory and nervine tonic. They are useful in gonorrhoea, sterility and general debility.

In addition, *Mucuna* is also traditionally used in various other applications like, dye^[5], treatment of pain and numbness of joints, and irregular menstruation^[6]. *Mucuna pruriens* (L.)DC. commonly known as Kivach, Alkusi, Cowhage, Kaunch, Velvet bean is an economically important medicinal plant found in

bushes and hedges and dry deciduous, low forests throughout the plains of India^[7,8].

Mucuna cochinchinensis locally known as Lyon bean is an annual twining herb with white or pale purple flowers and glabrescent pods. It is widely distributed in the tropics and subtropics and cultivated mostly in Bengal and Bihar region of India for its edible pods and seeds. The fleshy and tender fruits of the plant are valued as vegetable^[9].

The literature survey showed that in India *Kapikacchu* species are regularly used to treat patients and getting good results. But it is not clearly mentioned the effectiveness of the particular species. Comparative chemical analysis of all the species is not worked out till date. It is highly essential to find out and report the comparative analysis of all the *Mucuna* species. In view of the above mentioned literature survey, it was necessary to conduct comprehensive research work for comparative assessment of pharmacologically active principles and antioxidant activity of commonly occurring *Mucuna* sps. in India.

MATERIALS AND METHODS

Collection of plant material

Healthy, fresh and disease free plant material - seeds of three selected varieties of *Mucuna* sps (*Mucuna pruriens* (L.) DC., *Mucuna bracteata*, *Mucuna cochinchinensis* black seeds sp. and *Mucuna cochinchinensis* white seeds sp.) were collected from NRIBAS, garden, Pune and adjoining areas of Pune district, Kerala, Regional Research Institute (Ayurveda), Andaman and Nicobar.

Authentication and herbarium preparation

Plant materials were identified and authenticated with the help of floras and at least four voucher herbarium specimens of each species are prepared as per the accepted/ standard procedure and deposited in the Herbarium section of the NRIBAS, Kothrud, Pune.

Extraction of 4 *Mucuna* sps.

Mucuna pruriens, *Mucuna bracteata*, *Mucuna cochinchinensis* black seeds sp. and *Mucuna cochinchinensis* white seeds sp. were used for extraction. The crushed seeds powders of these 4 plants were immersed in water and the flasks were kept at 40°C in heating water bath overnight. The following day the extract was filtered through muslin cloth and kept in Lyophilizer (Labconco Freezone 4.5, at -50°C, 0.020mbar) to further concentrate it. The dried, powdered forms of these 4 extracts were preserved at dark and cold place to maintain the medicinal value. As part of phytochemical analysis L dopa (L-3, 4-dihydroxyphenylalanine) and other essential phytochemical levels were assessed in this.

L dopa quantitation

A thorough literature survey was done to find out the colorimetric method for quantitating the L-dopa content in the *Mucuna* seeds extracts [10]. A stock solution of 1000µg/ml of standard L dopa (L-3, 4-dihydroxyphenylalanine, Sigma-Aldrich) was prepared. From which a range of concentrations 100 to 10 µg/ml was made. The actual assay was performed in ELISA 96 wells plate. To each test solution 0.2% of freshly prepared isoniazid (INH, Sigma-Aldrich) solution was added and shaken well, allowed to stand for 10 min and then 5 ml of 20% sodium carbonate was added and mixed well. This solution was kept at room temperature for reaction and colour development. The absorbances of standard L-dopa solutions were recorded at 476 nm (Epoch BioTek). Based on the absorbances obtained for various concentrations of L dopa standard, calibration plot was made. The standard plot was used for quantitating the L-dopa content in 4 *Mucuna* sps.

Determination of proteins

The proteins were first treated with copper ion in alkali solution, and then aromatic amino acids in the treated sample reduce the phosphomolybdate phosphotungstic acid present in the Folin reagent. The

end product of this reaction has a blue colour. The amount of proteins in stem bark and leaves extracts was determined according to the method described by Lowry *et al.* [11]. The amounts of proteins were calculated by taking the absorbance at 700nm against the standard solution of 50% of bovine serum albumin (BSA).

Determination of flavonoids

When flavonoids react with aluminium chloride it forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. Total Flavonoids content in plant extracts was determined according to the method described by Khatiwora *et al.* [12]. The absorbance is measured at 415nm against the standard quercetin. Various concentrations of standard quercetin solution are prepared to make a standard calibration curve. The concentration of flavonoid in the test sample was calculated from the calibration plot.

Total Phenolic Content (TPC)

Total phenolic content (TPC) from extracts were quantified using Folin-Ciocalteu's method adapted to 96-well microtitre plate with minor modifications [13]. 100 µl of 1:4 diluted Folin - Ciocalteu's phenol reagent, 2N (Sigma - Aldrich) in distilled water was added to 20 µl of serially diluted (10 - 1000 µg/ml) lyophilized plant extracts and standard Gallic acid dissolved in distilled water. After 5 min incubation at room temperature, 80 µl of sodium carbonate (75 g/L) were added to each well. The 96 - well plate was slightly shaken and incubated for 30 min at room temperature in the darkness. The absorbance was measured at 735 nm using ELISA plate reader (Epoch Biotek). The assay was repeated thrice and Total phenolic contents (average of three) were expressed as Butylated Hydroxy Toluene equivalent per gram of lyophilized extract.

Inhibition of DPPH radical

The free radical scavenging activity of *N. Nimmoniana* extracts was measured by 1; 1-diphenyl-2-picryl-hydrazil (DPPH) as a method described by Blois [14]. This assay measures the capacity of the extract to donate hydrogen or to scavenge free radicals. DPPH radical is stable free radical and on reacting with an antioxidant compound which can donate hydrogen, it is reduced to diphenyl picryl hydrazine (DPPH). The change in colour from deep-violet to light yellow was measured spectrophotometrically at 517 nm for various concentrations against the standard Butylated Hydroxy Anisole (BHA).

Reducing power/Ferric reducing antioxidant potential (FRAP) assay

The reducing power of plant extracts was determined according to the method of Oyaizu [15]. About 200 mg of plant extract in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A

portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 g for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and Ferric chloride (FeCl₃) (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Butylated Hydroxy Toluene (BHT) was used as the reference material. All the tests were performed in triplicate and the graph were plotted with the average of three observations.

RESULTS

Mucuna cochinchinensis having black seed was found to have highest L-dopa content of 190.7156 µg/ml, and it was much greater than other 3 *Mucuna* sps. under the study, it was almost two fold of that of the content of *Mucuna cochinchinensis* white seed, *Mucuna pruriens*, *Mucuna bracteata* was having the least L-dopa content (Table 1). The essential chemical

components of plant namely protein, total phenols and flavonoids were quantitated using standard protocols reported. The observations of concentration of protein, flavonoid and total phenolics and antioxidant potential using FRAP and DPPH assay are illustrated in Table 2 and Figure a-e. *Mucuna cochinchinensis* with black seed extract had highest protein and total phenolic content among all the extracts, which is more than 10 fold higher content of total phenolics and proteins in comparison to the rest 3 extracts. *Mucuna bracteata* aqueous extract had highest flavonoid content (104.88 µg/ml). In DPPH radical scavenging assay extract of *Mucuna cochinchinensis* black seed had highest % inhibition activity at 1000µg/ml of extract (99.856%). In FRAP assay *Mucuna pruriens* extract showed highest potential to reduce the ferric ions (equivalent of 55.74 µg BHT).

Table 1: The quantities of L-dopa content observed in 4 species of *Mucuna* are represented in µg/ml.

Sl. No.	Name of the extract (aqueous-1 mg/ml)	L dopa content (µg/ml)
1.	<i>Mucuna cochinchinensis</i> black seed	190.7156 ± 4.2161
2.	<i>Mucuna cochinchinensis</i> white seed	96.2282 ± 3.9161
3.	<i>Mucuna pruriens</i>	77.6826 ± 3.1232
4.	<i>Mucuna bracteata</i>	42.1153 ± 2.8933

Table 2: Assessment of few essential phytochemicals such as protein, flavonoid and total phenolics and antioxidant potential using FRAP and DPPH assay

Sl. No.	Name of chemical test/assay	<i>M. cochinchinensis</i> black seed extract	<i>M. cochinchinensis</i> white seed extract	<i>M. pruriens</i> extract	<i>M. bracteata</i> extract
1	Total Phenolic Content (µg BHT/1000 µg of extracts)	233.085 ± 7.8219	41.06 ± 2.8151	7.336 ± 1.2312	9.502 ± 1.1009
2	Protein assay (µg protein/1000 µg of extracts)	887.28 ± 7.9191	6.851 ± 1.1092	8.512 ± 1.2592	7.543 ± 1.3128
3	Flavonoid assay (µg flavonoid/1000 µg of extracts)	22.43 ± 3.1259	66.13 ± 2.8152	33.84 ± 4.1266	104.88 ± 7.9128
4	DPPH assay (% Inhibition/1000 µg of extracts)	99.856 ± 5.1962	99.773 ± 6.9768	92.9135 ± 6.2851	97.1691 ± 6.9271
5	FRAP antioxidant assay (µg BHT/1000 µg of extracts)	45.772 ± 3.2135	47.103 ± 5.9562	55.74 ± 4.2791	40.3715 ± 3.7122

DISCUSSION

Almost all the species are reported to contain L-3,4-dihydroxy phenylalanine (L-Dopa), a non-protein amino acid that acts as precursor for the neurotransmitter dopamine, used in the treatment of Parkinson's disease [4, 16,17, 18]. L-DOPA plays important role in spermatogenesis and recovery of endocrine axis. The effect of L-DOPA were not comparable to *Mucuna pruriens* however, it suggested that L-DOPA as one of the active principle in *Mucuna pruriens*. The production of gonadotropin releasing hormone was activated by stimulation of the hypothalamus and forebrain, by L-DOPA. This stimulates anterior lobe of pituitary for secretion of FSH and LH which activates thuriens is largely due to L-DOPA [19]. The L-DOPA induced facilitation of sexual behavior in the male rats were mediated by dopamine. The effects of L-DOPA were observed in elements preceding and during the copulatory act as described by Malmnas [20].

L-DOPA possesses antioxidant activity which was supported by *in-vitro* antioxidant assays [21]. The antioxidant and free radical scavenging activity of Dopamine a metabolic product of L-DOPA was found to be highly significant [22, 23]. This indicates that the antioxidant activity of the aqueous extracts of all the *Mucuna* sps. may be due to the presence of L-Dopa content in its seed.

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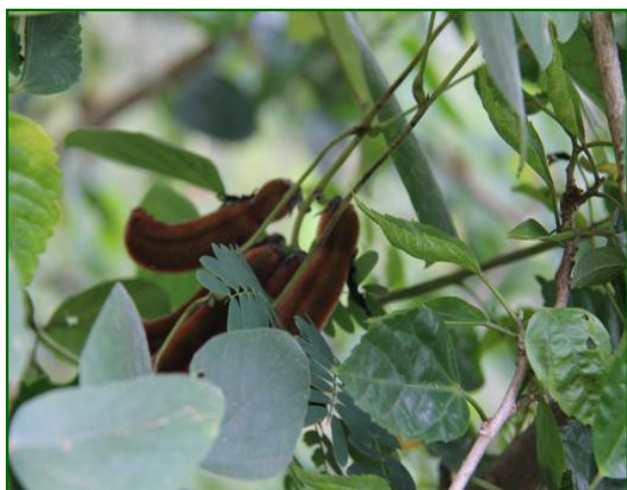
Mucuna sps. Photographs



Pods of *Mucuna cochinchinensis* (Lour.) A.Chev. black seeds sp.



Pods of *Mucuna cochinchinensis* (Lour.) A.Chev. white seeds sp



Pods of *Mucuna bracteata* DC.



Pods of *Mucuna pruriens* (L.) DC.



Flowering twig of *Mucuna pruriens* (L.) DC.

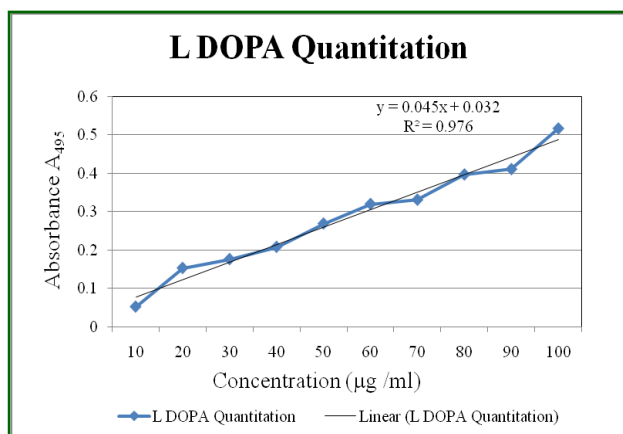


Figure a: The standard plot of L-dopamine

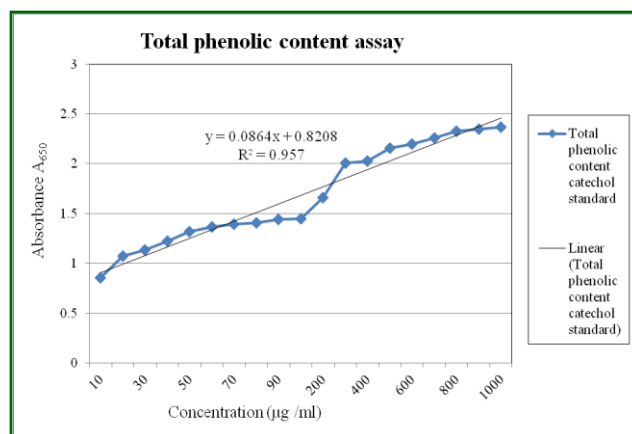


Figure b: The standard plot of total phenolic content assay

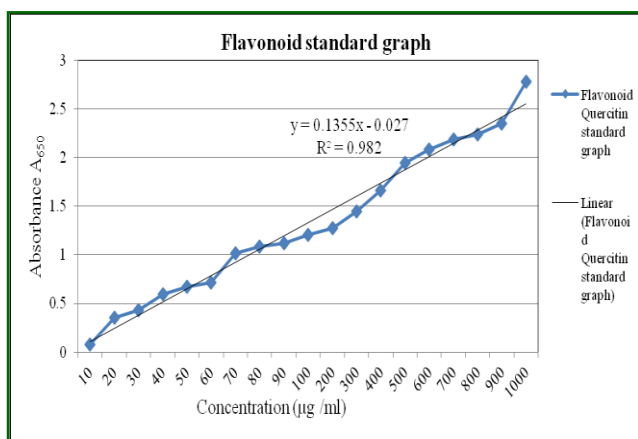


Figure c: The standard plot of flavonoid assay

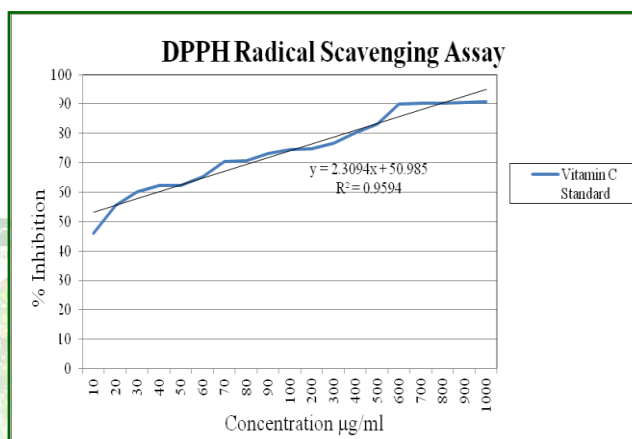


Figure d: The standard plot of DPPH radical scavenging assay

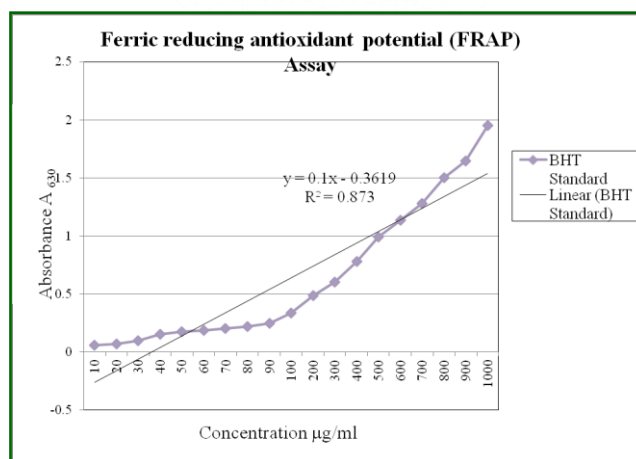


Figure e: The standard plot of FRAP assay