EFFICACY AND POTENCY SCREENING OF AVAILABLE FIELD AND MARKET SAMPLES OF KAPIKACHU SEEDS (*MUCUNA* SPECIES) ON THE BASIS OF PHARMACOGNOSTICAL, PHYTOCHEMICAL AND VRISHYA (SPERMATOGENESIS) ACTIVITY

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March 2016

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CERTIFICATE

This is to certify that the thesis entitled "**Efficacy and Potency Screening of Available Field and Market Samples of Kapikachu Seeds (*Mucuna* species) on the Basis of Pharmacognostical, Phytochemical and Vrishya (Spermatogenesis) Activity**" which is being submitted herewith for the award of degree of Vidyavachaspati (Ph.D.) in The Late Vd. P. G. Nanal Department of Ayurveda, Tilak Maharashtra Vidyapeeth, Pune is a result of original research work completed by **Vd. S. N. Murthy** under my supervision and guidance. To the best of my knowledge and belief, the work incorporated in this thesis has not formed the basis for the award of any degree or other similar title of this or any other Vidyapeeth or examining body upon him.

Place: Pune

Date:

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Vd. Yogini Kulkarni

DECLARATION

I hereby declare that the thesis entitled "**Efficacy and Potency Screening of Available Field and Market Samples of Kapikachu Seeds (*Mucuna* species) on the Basis of Pharmacognostical, Phytochemical and Vrishya (Spermatogenesis) Activity**" completed and written by me has not previous been formed as the basis for the award of any degree or other similar title of this or any other Vidyapeeth or examining body.

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Abbreviations

A.H.	:	Ashtanga Hridaya
A.Ni	:	Astanga Nighantu
A.S	:	Ashtanga Samhita
API	:	Ayurvedic pharmacopia of India
B.Ni	:	Bhavaprakasha Nighantu
B.Ra	:	Bhaishajya Ratnavali
C.D.	:	Chakradatta
C.S.	:	Caraka Samhita
Chi	:	Chikitsasthana
D.Ni	:	Dhanvantari Nighantu
G.Ni	:	Gada Nigraha
H.Sa	:	Harita Samhita
K.Ni	:	Kaideva Nighantu
Ka	:	Kalpasthana
M.Ni	:	MadanapalaNighantu
Ni	:	Nidanasthana
P.Ni	:	Priya Nighantu
R.Ni	:	RajaNighantu
S.Ni	:	SodhalaNighantu
S.S	:	Suruta Samhita
Sa	:	Sareerasthana
Si	:	Sidhisthana
Su	:	Sutrasthana
Ut	:	Uttarasthana
Utt	:	Uttarakhand
V.S.	:	Vangasena samhita
Vi	:	Vimanasthana
Y.R	:	Yogaratnakara

Abstract

Infertility in males is one of the major concern areas in the present socio-environmental condition, where lifestyle, food habits of individuals are playing major role. The present study is aimed to find out the most effective species of *Mucuna* (Kapikachu), which is being used by many Ayurvedic scholars since ages for the treatment of Infertility. In the present available conditions there were 3 species of *Mucuna* being used as Kapikachu for treating the conditions related to low sperm count or presence of abnormal sperms.

In the present study three species of *Mucuna v*iz. *Mucuna pruriens* (L.) DC., *Mucuna c*o*chinchinensis* (Lour.) A.Chev (White seed), and *Mucuna cochinchinensis* (Lour.) A.Chev. (Black seed) were considered for screening to determine their efficacy and potency on basis of their *vrishya* (Spermatogenesis) activity. Pharmacognostical and Phytochemical studies of these three seed samples were carried out to develop the identification keys. Seed samples were collected from their native source and got authenticated by the experts of Agharkar research institute, Pune, as per the standards.

The detailed pharmacognostic study was carried out with anatomical studies of seed along with morphology. The powder studies with various parameters of these seed samples and including phytochemical studies such as Loss on drying, Ash values, Different extractive values, Qualitative and Quantitative analysis, Elemental analysis were also carried out. Experimental results of these studies have provided a clear picture about drug profile of Kapikachu characterized by presence or absence of important phytochemical constituents not only quantitatively but qualitatively also, which helped in development of identification keys for these seed samples.

In the process of developing the identifying genetic markers for all these, three, study samples, the most authentic and accurate method *i.e.* DNA fingerprinting through RAPD technique was carried out and a large similarity values were reported by RAPD markers. The 25 tested primers yielded approximately 250 scorable bands with an average of three bands per primer which showed polymorphism. 60% polymorphism was observed in *M. cochinchinensis* and 92% in *M. pruriens*. Most

used primers in the analysis showed amplification for all the samples whereas primer number 4, 18 and 24 did not show any bands in the RAPD profile.

As a part of this studies L-dopa content and antioxidant potential of these three seed samples were carried out using different assays *viz*. Total phenolic content, flavonoid assay, DPPH assay, FRAP antioxidant assay. During experimental analysis biochemical analysis for serum testosterone, Luteinizing Hormone, Follicle Stimulating Hormone, and estradiol levels in treated and control rats.

To determine the potent species among all these three seed samples with respect to their *Vrishya* (Spermatogenesis) activity, water extract of all these three seed samples were screened for spermatogenetic activity in Sprague Dawley rats at various doses. L-dopa was used as standard in this study. Treatment with standard drug at 20 mg/kg and *Mucuna cochichinensis* (Black seed) and *Mucuna pruriens* at 400 mg/kg body weight for 30 days induces significant increase in sperm count, motility and morphology of sperms ($p< 0.05$).

Apart from spermatogenetic studies acute and sub acute toxicity studies were also carried out on rats. In this study *M. cochinchinensis* (Black seed), *M. pruriens* and *M. cochinchinensis* (White seed), were found to be safe up to 2000 mg/kg body weight when administered orally to rats. The NOEL of *M. cochinchinensis* (Black seed) was found to be 1000 mg/kg body weight when administered orally for 28 days.

Chapter 1: Introduction

Health is the supreme foundation for wealth, enjoyment and salvation. Poor health or diseases are the impediments for good life, and progress of humanity. The sage Bharadwaja, who brought Ayurveda as a remedy to these impediments from Lord Indra, in the form of Hetu jnan (Causes and Etiology), Linga jnan (Knowledge of signs and symptoms), and Oushadi jnan (Knowledge of medicines / Dravyaguna). Out of these three principles of knowledge, Ayurveda developed into eight specialties. Thus, Dravyaguna has become an integral part of all the branches of Ayurveda (Dhyani,1994).

Dravyas (Substances) act by virtue of its properties such as Rasa, Guna,Virya, Vipaka, Prabhava. Pancha Mahabhutas are the contributing factors of dravya and its gunas. These are also called as karana dravyas. Rasa, Guna,Virya,Vipaka, Prabhava, are formed out of permutations and combinations of these elementary substances. The knowledge of rasadi panchaka is important to understand dravya karmukata (modus operandi) of Ayurvedic drugs. According to Caraka, drugs act by virtue of their nature as substance (dravya prabhava), or by virtue of their properties (guna prabhava) or by virtue of both (dravya guna prabhava) (Dhyani, 1994).

Ayurveda, the ancient science of life believes in the philosophy of not only treating the diseases but also to help in maintaining the healthy state of every individual. Vrishya chikitsa is one of the eight branches of Ayurveda which is devoted to the treatment of infertility, impotence and other diseases related to reproductive system in males. Vrishya (Aphrodisiac) therapy helps in nourishing the body tissues of an individual with a target of improving the quality of germinative tissues. Shukra is considered as most important and essence of all body tissues. In this way, the core nutrients / elements of Majja dhatu helps in production of Shukra dhatu (semen). According to Ayurveda, Shukra dhatu is pervading all over the body in indistinguishable manner like ghee in the milk. This is invisibly present in all over body gets ejaculated in the visible form when men indulge in sexual activities. In Ayurveda, Shukra is described to be present even in children like fragrance in buds and it is visible when they attain adulthood. Shukra is also present in women, but this

is not useful for garbhotpatti. Since Shukra dhatu is the end product of all dhatus, it has to be protected and maintained very carefully by consuming healthy food and by practicing healthy sexual life. According to the scholars of Ayurveda, a part of Shukra under goes further dhatwagni paaka and produces Ojas. This Ojas becomes a part of every dhatu and provides strength and stability to them (bodily tissues).This entire process of metamorphosis of dhatus from Rasa to Ojas takes 6 days acc to Caraka, and one month according to Sushruta (C.S.chi 15/16, S.S.su.14/10, A.H.sha.3/62-63, C.S.vi.5/3).

As mentioned earlier, the healthiness of Shukra depends on the healthy state of Shukragni, which, if is in proper state, helps in transforming majja dhatu into Shukra. If Shukragni is affected by vitiated Vata dosha, then it produces the semen which is thin, frothy, mild red in colour, scanty, accompanies with pain and difficult when ejaculated. According to Caraka this kind of Shukra doesn't have potency for fertilization. Acharya Sushruta used the term "*Vaata varna vedanam*" in this reference. If this Shukragni is affected by vitiated Pitta dosha, the resultant Shukra is in mild yellow or blue in colour, results in burning sensation when ejaculated. Acharya Sushruta used the term "Pitta varna vedanam". If this Shukragni is affected with vitiated kapha dosha, the Shukra which is formed is more in quantity, and thick in consistency. Sushruta used the term "Shleshma varna vedanam". The Shukra which is affected with all vitiated tridoshas, has smell of urine and stool (A.S.sha.1/13, C.S.chi.30/139-145, S.S.sha.2/4).

1.1 Need of the study:

In the present socio-environmental conditions, life style, stress and food habits have become a major factor in causing infertility in males. Internationally conducted several studies suggest that there is a direct link between junk food with high Trans fats and poor sperm health in young men. A study conducted by Harvard university and University of Murcia in Spain in October 2011, on young men ages between 18 to 22, who were in good health and had no other issues that could negatively affect sperm health, have shown the sperm less likely to survive inside womb.

Another study carried out on a large sample of 1683 patients, and published in Reproductive and Endocrinology (2012), was evaluated based on Motile Sperm Organelle Morphology Examination (MSOME) and World Health Organization (WHO) criteria. This study has shown no single parameter that affect sperm health directly. But combinations of high Body Mass Index (BMI), regular coffee intake, low ejaculatory frequency and sexual abstinence have a negative impact on sperm motility. The concluding findings of this study showed that different adverse life style factors could be detrimental to sperm health, motility and amount of cellular fluid in the sperm head.

Keeping the importance of Shukra and its related disorders in the light of above mentioned studies, the present study is designed with the drug Kapikachu to work upon its role in improving the quality and quantity of Shukra (semen), with its effect on endocrine axis as primary target along with study of its contents, and micro macro differences of various samples of Kapikachu that are being used by Ayurvedacharyas for treating their patients.

Kapikachu, a well known vrishya drug mentioned in Ayurvedic texts right from Caraka and Sushruta samhita period as a drug for balya, and advocated to use for vajikarana chikitsa. Caraka mentioned it in Balya and Pureesha viranjaneeya groups as Rishabhi, Kachura. He also made this drug as an ingredient in the formulations like Maha mayura ghrita and Amrita prasha ghrita. With the synonym of Markati this drug was used in the treatment of Unmada by Caraka. Sushruta for the first time used this drug for vajikarana chikitsa. During nighantu period Bhavamishra used this for vata samana chikitsa and mentioned Vrishya properties of this drug can be obtained by using beeja churna. According to Sodhala excessive use of beeja churna causes Mada (intoxication) or shows some toxic effects.

Infertility in males is generally refers to impaired sperm quality in terms of motility and reduced count. Azoospermia, complete absence of sperm or oligospermia low concentration of sperm are usually idiopathic in nature. Some times may be the consequence of longstanding hormonal imbalance. In such cases more than one factor is involved in causing infertility. Oligospermaesthenia, oligoasthenaspermia, oligoasthenoteratospermia are the other terms where low sperm count is associated

with poor sperm motility. In about 30% of infertile men no cause is diagnosed for their decrease in sperm concentrations, and quality. Inflammatory mediators which were reportedly released by mast cells are appearing to be one of the reasons for suppression of sperm motility.

Reasons for oligospermia can be classified into three categories based on their level of pathology, they are 1. Pre testicular 2.Testicular 3. Post testicular causes.

1. Pre testicular: Hypogonadism, Drugs, Alcohol, Smoking, Medications including androgens.
2. Testicular: These factors refer to the conditions where the testes produce semen of poor quality despite of adequate support of hormones, they are like genetic defects on the chromosome, Klinfelter syndrome, neoplasm (Seminoma), Varicocoele , Cryptorchidism, trauma, Hydrocoele, Mumps.
3. Post testicular: Vas deferens obstruction (vasectomy) or absence, infection (Prostitis, STD), ejaculatory related issues.

According to Botanists in India Kapikachu is identified as *Mucuna pruriens.* (L.) DC. There are 15 *Mucuna* species reported in India, out of which 4 plants are being used as Kapikachu, by Ayurvedic practitioners depending on their availability in their areas. Majorly they are

1. *Mucuna pruriens* (L.) DC.
2. *Mucuna cochinchinensis* (Lour.) A.Chev. (Black and white seed).
3. *Mucuna monosperma* DC. ex Wight
4. *Mucuna gigantea* (Willd.) DC.

During the course of literature survey, it is found that in India, Ayurvedic practitioners are using various seeds of *Mucuna* sps, as Kapikachu for the treatment of infertility, general debility, nervine disorders, Parkinson’s disorder, and getting positive results.

This study was taken up, since no comparative study was carried out in respect of chemical composition of all the species, effectiveness of their seed powder on

infertility, spermatogenesis, L- dopa content, DNA (Deoxyribose Nucleic Acid) fingerprinting using Random Amplification of Polymorphic DNA (RAPD) technique. Hence, studies were carried out to compare the effectiveness of seed powder as mentioned in Ayurvedic texts, on spermatogenesis activity (Vrishya) also along with their chemical composition, L- dopa content and RAPD, of available field and market samples of Kapikachu (*Mucuna* sps). The present study is mapped with the following aims and objectives.

1.2 Aim:

To determine efficacy and potency of available field and market samples of Kapikachhu seeds (*Mucuna* sps) on the basis of Pharmacognostical, Phytochemical and Vrushya activity (Spermatogenesis)

1.3 Objectives of the study:

1. To study the comparative pharmacognostic, phytochemical contents, properties of seed samples collected from fields and markets.
2. To develop identification keys of selected *Mucuna* samples on the basis of pharmacognostic, phytochemical and DNA fingerprinting using RAPD technique.
3. To find out the potent species on the basis of phytochemical screening and Vrushya activity (Spermatogenesis). Hormonal changes and anti-oxidant property in Albino rat (Sprague Dawley strain) with selected species of *Mucuna*.

1.4 Plan of study:

The present research work has been planned out in three phases; *viz.*, Conceptual, Analytical, Experimental study.

Chapter 2: Drug Review

The knowledge of drug (medicinal plants) goes back to the prehistoric days. In Vedic period man had close contact with the environment, in specific with plants as he had dependent on them not only for his regular requirements but also for agricultural and domestic use. Plants played an important role in their religious ceremonies, rites and sacrifices in different forms. Apart from all these, plants were also used as drug (Bheshajya-medicine) to cure / alleviate many diseases of human kind.

2.1 Literature Review of Kapikachu

Vedas as old as 6000 BC, the oldest repository of human knowledge displays the material and rationale knowledge of plant kingdom and it's exploitation for the benefit of humanity. It is the oldest extensive source of drugs which can be defined as oldest drug stock of ayurveda. Oushadhi sukta of Rigveda is the authoritative document of plant knowledge of that period.

The Atharva veda (1500 BC) is having more descriptive picture of medicinal plants used in many diseases. The number of drugs mentioned in Atharva veda are more than Rigveda as there is an addition in the knowledge by advance of time, and that is the reason for the presence of more number of plants in Atharva veda than other Vedas. Although the different branches of Ayurveda have their seeds in Vedas, the word Ayurveda is conspicuously absent in Vedas.

In Vedic literature Kapikachu is not mentioned. Even in brihatrayi the term Kapikachu is not used widely. All 3 Samhitas (Caraka, Sushruta, Vagbhata) described this plant majorly with the terms like Atma gupta, Svayam gupta, Markati etc. Even though the term Kacchura was used by Caraka, (C.S.Su.4), according to Vd.Thakur Kacchura can't be attributed to Kapikachu, but it can be considered for Duralabha (Sastry, 2005).

2.1.2 Samhita kala

Caraka Samhita (1000 BC – 4th AD)

Among the existing old medical texts of India, Caraka samhita is considered as the oldest one. This samhita was written / composed by Agnivesa (1000 BC), redacted by Caraka (2nd BC), and reconstructed by Dridhabala (4th AD). This text has mainly dealt with different diseases and types, and their management with different medicines. Which means this samhita is mainly focused with the medical management of diseases that is Kayachikitsa branch of Ayurveda. Here in this text Acharya Caraka has mentioned Kapikachu with the name of Rishabhi in balya dasaimani varga, as Kachura in Pureesha viranjaneeya dasaimani varga, and as Rishyaprokta in madhura skanda dasaimani varga kashayas. He also used the term Adhyanda for (C.S.Chi.3/266) Kapikachu.

Table 2.1: Preparations and indications of Kapikachu in Caraka Samhita

S.No.	Name of the preparation	Indication	Reference
1	Bramha rasayana	Medho vridhi	C.S.Chi.1/1-58
2	Vrisya pupalika	For Vrishya action	C.S.Chi.2/3-15
3	Maha paisachika Ghrita	Fever, Insanity, Seizures	C.S.Chi.9/45-48
4	Swadamstradi Ghrita	Vata Pitta hara	C.S.Chi.11/44-47
5	Tryusanadya Ghrita	Kasa, Jwara, Gulmahara	C.S.Chi.18/39-42
6	Medicated Ghrita	Svasa, Kasa,Grahani	C.S.Chi.26/80-82
7	Jivantyadi Anuvasana Yamakam	Brimhana, Vatapitta nasaka, Bala, Sukra Agnivardhaka.	C.S.Si.4/9-11
8	Vrishya yoga pupalika	Vrishya	C.S.Chi.2/2

Sushruta Samhita (1000 BC – 4thAD):

This Samhita is composed by Vridha Sushruta which is chiefly dealt with different aspects of Shalya chikitsa (surgical and parasurgical mode of treatment) in

Ayurveda. This text is redacted by Sushruta in 2nd AD. Later, it was probably revised by Acharya Nagarjuna, the greatest scientist in Bhudhist period *i.e* during 5th AD. In the existing form it appears to be much later than Caraka samhita, because its paatha sudhi was done by Chakradata in 10th century. In this Samhita, Kapikachu is mentioned in Vidari gandhadi and Vata samsamana ganas. Sushruta has used Kisimbivalli (S.S.Su. 46/46) and Languli (S.S.Su. 19/29) as synonyms for Kapikachu. For the first time in this samhita properties of Kapikachu beeja were mentioned separately (S.S.Su.46/36).

Table 2.2: Preparations and indications of Kapikachu in Sushruta Samhita

S.No	Name of the Preparation	Indication	Reference
1	Vrishya yog	Vrishyata	S.S.Chi.26/30-35
2	Jeevantyadi Anuvaasana Yamakam	Vrishya, Deepana.	S.S.Chi.37/23-26
3	Kapikachu churna	Pitta, kapha vidaghda drishti	S.S.Ut.17/7
4	Kapikachu Madhuyashti yoga	Vamana hara	S.S.Ut.49/29
5	Kapikachu,Ikshurasa, Pippali Ghrita yoga	Mutraghata, Vandhyatwa	S.S.Ut.58/53-57

Ashtanga Sangraha:

This Samhita is composed by Vridda Vagbhata in 6th AD. In this text Kapikachu is mentioned in Durvadi and Vidaryadi ganas (A.S.Su.16/2).

Table 2.3: Preparations and indications of Kapikachu in Ashtanga Sangraha

S.No	Name of Preparation	Indication	Reference
1	Kapikachu sidda Ghrita	Nasal drops to Garbhini	A.S.Su.29/11 and A.S.Su.7/31
2	Kapikachu mardana	Sanyasa/ Madatyaya	A.S.Chi.9/125
3	Nagabala ghrita	Kshata khaya, Pittarakta	A.S.Chi.5/69
4	Amrita taila	Vata vyadhi chikitsa, Agnideepana	A.S.Chi.23/72
5	Taila	Vatavyadhi chikitsa	A.S.Chi.23/86
6	Ghrita	Kshta Kshaya	A.S.Chi. 5/53

7	Kashaya preparation	Pittaja Chardi	A.S.Chi.8/19
8	Raja Yapana Basti	Aphordisiac / Vrishya	A.S.Ka.5/23
9	Yapana Bati	Vrishya / Vitality	A.S.Ka.5/34
10	Medicated Oil	Vata Pitta diseases related to sukra, Rajas.	A.S.Ka.5/49
11	Medicated Oil	Abhyanga	A.S.Ut.1/80
12	Maha kalyana Ghrita	Sannipaata vyadhis, Sthoulya	A.S.Ut.9/30
13	Maha paishachaka ghrita	Unmada, Apasmara, Sannipata Jawara	A.S.Ut.9/32
14	Medicated Oil	Paalitya Chikitsa	A.S.Ut.28/46
15	Maha mayura Ghrita	Cures Different Indriya vyadhis, Sirorogas.	A.S.Ut.28/73
16	Ksheeraa preparation	Vrishya , Visha	A.S.Ut.48/57
17	Saradi Ghrita	Vrishya	A.S.Ut.50/26,30
18	Beeja Churna with Ksheera	Vrishya	A.S.Ut.50/39,47.
19	Svayam Gupta Churna Yoga	Vrishya	A.S.Ut.50/58,60
20	Medicated Ghrita	Vrishya	A.S.Ut.50/62
21	Kapikachu molasidda Kheera & Ghee	Vrishya	A.S.Ut.50/64
22	Kapikachu sidha Poopalika with Ghrita	Vrishya	A.S.Ut.50/65, 68,69
23	Padalepa Yoga	Vrishya	A.S.Ut.50/106, 108

Ashtanga Hridaya:

This samhita is composed / written by Laghu Vagbhata during 7th AD. In this text also Kapikachu is mentioned in Vidaryadi Gana (A.H.Su.-15/9) both Vagbhatas have also mentioned different yogas of Kapikachu keeping its Vrshya/ Vajikarana activity in mind.

Table 2.4: Preparations and indications of Kapikachu in Ashtanga Hridaya

S.No	Name of the preparation	Indication	Reference
1	Medicated Ghee	Cough	A.H.Chi. 3-38
2	Amrita prasa ghrita	Cough,Hiccup	A.H.Chi 3/94-101
3	Svadamshtradi Ghrita	Vata pitta Hrid sula, Dysuria.	A.H.Chi.3/102-103
4	Agastya Rasayana (Hareetaki Rasayana)	Rasayana,	A.H.Chi. 3/127-132
5	Vashishta (Hareetaki) Rasayana	Rasayana,	A.H.Chi. 3/133
6	Jeevantyadi Kalpa (Vaata pitta hara kalpa)	Asthapana vasthi (Retention enema)	A.H.Ka.4/60
7	Medicated oil	Lepana for Karna pali Sandhana	A.H.Ut.40/17
8	Beeja churna, masha flour with Ksheera	Vrishya	A.H.Ut.40/31
9	Kapikachu beeja churna, svadamshtra, ikshuras, satavari with milk	Vrishya	A.H.Ut.40/34
10	Kapikachu as diet	Vrishya	A.H.Su.6/22
11	Vidaryadi Ghrita	Vrishya	A.H.Ut.2/21,31,1/23

2.1.3. Laghutrayi and Chikitsa granthas period

Like in Samhitas, Kapikachu is mentioned in chikitsa granthas as Kapikachu and also by its synonyms *viz.* Atmagupta, Kandura etc, in the management of several diseases.

Chakradatta (Composed by Chakrapani in 11th AD):

In this text Kapikachu is mentioned for different diseases apart from its vajikarana activity along with other drugs.

Table 2.5: Preparations and indications of Kapikachu in Chakradatta

S.No	Preparation	Indication	Reference
1	Balamuladi Nasya Chatushtayam	Vatavyadi	C.D.22-27
2	Mashadi Kashaya (Maha Baladi kwath)	Vatavyadi	C.D.22.28
3	Svaguptadi leha	Mutraghata	C.D.33/17,18
4	Svayan guptadi churna	Vrishya	C.D.67/6
5	Godhumadiya ghrita	Vrishya	C.D.67/26-36
6	Gokshuraadi churna	Vrishya	C.D.66/26-34

Vangasena Samhita:

Table – 2.6: Preparations and indications of Kapikachu in Vangasena Samhita

S.No	Preparation	Indication	Reference
1	Brihad Aswagandhadhi ghrita	Vajikarana purpose	V.S.84/22-27
2	Medicated Ghrita	Vajikarana purpose	V.S.84/65-66
3	Gokshura Kapikachu beeja sidha ksheera	Vajikarana purpose	V.S.84/73
4	Masha Ghrita	Vajikarana purpose	V.S.84/75-79
5	Godhumaadi Ghrita	Vajikarana purpose	V.S.84/80-89
6	Jeevanti Yamaka	Anuvasana, Nasya, Panaartha, Urdhva jatrugata roga.	V.S.84/93-95

Yoga Ratnakar: In this text Kapikachu is described in Uttarakhanda,Vaajikarana adhyaya.

Table 2.7: Preparations and indications of Kapikachu in Yoga Ratnakar

S.No	Preparation	Indication	Reference
1	Vanari gutika	Vajikarana	Y.R.Utt.11/27-31
2	Kesar paka	Vajikarana	Y.R.Utt.11/49-56
3	Rati modaka	Vajikarana	Y.R.Utt.11/57-60
4	Kameswara modaka	Vajikarana	Y.R.Utt.11/68
5	Kameswaar samdi paka modaka	Vajikarana	Y.R.Utt.11/77-83.
6	Madana kamadeva rasa	Vajikarana	Y.R.Utt.11/117
7	Rati vallabha pugapaka	Vajikarana	Y.R.Utt.11/61-67

8	Satavaryadi churna	Vajikarana	Y.R.Utt.11/146
9	Kapikachu paka	Vajikarana	Y.R.Utt.11/156-160

Bhaishajya Ratnavali (Composed by Govinda Das In 18th cent AD):

Table 2.8: Preparations and indications of Kapikachu in Bhaishajya Ratnavali

S.No	Preparation	Indication	Reference
1	Svayam gupta Ikshuraka yoga	Dhaatukshaya	B.R.70/14
2	Gokshuradi yoga	Vajeekarana purpose	B.R.70/15
3	Godhumadya ghritam	Vrishya purpose	B.R.70/18
4	Brihadaswagandha ghritam	Vrishya purpose	B.R.70/19
5	Bricchatavari modaka	Vrishya purpose	B.R.70/21-24
6	Rativallabha modaka	Vrishya purpose	B.R.70/25
7	Mahakameswara modaka	Vrishya purpose	B.R.70/26
8	Kamagni sandipana modaka	Vrishya purpose	B.R.70/27
9	Manmadhabhra rasa	Vrishya purpose	B.R.70/29
10	Mophara-Mopharava (Yavana krit yoga)	All diseases, consumed by kings Vrishya purpose.	B.R.70/30

Gada Nigraha:

Table 2.9: Preparations and indications of Kapikachu in Gada Nigraha

S.No	Preparation	Indication	Reference
1	Mashadi Ghritam	Vaajikaranartha	G.Ni.9-12
2	Vajikaropayas Yoga	Vaajikaranartha	G.Ni.14
3	Vajikaram Masha parnyadi churnam	Vaajikaranartha	G.Ni.16
4	Vajikaram Swaguptadi churnam	Vaajikaranartha	G.Ni.19
5	Vajikaram Swayamguptadi churnam	Vaajikaranartha	G.Ni.29
6	Vajikaram Swayamguptadi Modakam	Vaajikaranartha	G.Ni.31-5, 37.
7	Vajikaram Sharmuladi Yoga	Vaajikaranartha	G.Ni.40-42
8	Vajikari Godhumadi Pupalika	Vaajikaranartha	G.Ni.43-44
9	Vajikaram Ikshurkadi Churnam	Vaajikaranartha	G.Ni.49.

Harita Samhita:

Table 2.10: Preparations and indications of Kapikachu Harita Samhita

S.No	Preparation	Indication	Reference
1	Visadi churna	Veerya vardhaka, Vajikara	H.Sa.47/9
2	Guduchyadi Churna	Vajikara	H.Sa.47/13,14

2.1.4. Nighantu kala

The word Nighantu is derived from the term "*NIGAMA*" etymology of nigama is that which brings out the extremely concealed or secrete meaning of words in systemic way is called nigama. *(Nighoodam artham gamayanti jnaapayanti iti nigamah*). Yagnyavalka wrote the nirukta to point out the concealed meanings of the words of Vedas. Thus Nigama, Nirukta, and Nighantu are synonymous. Among ayurvedic scholars also many have adopted this technique of explaining the charecters of medicinal plants in the form of synonyms, later on the descriptive details of technical properties; indications, actions etc were also included in Nighantus along with synonyms.

At this juncture, it is apt to mention a well known verse from Rajanighntu, which explains the importance of Nighntus. It declares a physician without the knowledge of Nighnatu is like scholar without the knowledge of vyakarana (grammar) and a soldier without weapons – all these are being laughed at in the world. This explains the knowledge of Nighantus is indispensible for a physician.

Astanga Nighantu (8^{th} AD):

Acharya Vahata has described maximum group of drugs in this text based on their classification of Astanga sangraha and Astanga Hridaya. To Kapikachu he has used the synonyms like Markati, Atmagupta, Kapikacchura, and Vrishyabeeja.

Dhanvantari Nighantu (10^{th} -13^{th} AD):

In the beginning of this text the author after paying homage to lord Dhanvantari, mentions that the name of this text is as Dravyavali. At the end of this text Dravyavali the author described the drugs of dravyavali with their synonyms. After describing drugs and their synonyms, they were again described along with their properties and actions. Here the author explains many drugs which are not described

in Dravyavali. It seems that these drugs came into existence in 13thAD. Dravyavali is quoted by Chandrata (10th AD), in his book "Yoga ratna samuchaya". Hence it looks that the descriptive details of rasa guna virya vipaka are inserted by some other person who could be Mahendra bhogika.

In this book Kapikachu is mentioned in Guduchyadi varga. In this text, Kapikachu is mentioned with the synonyms like Kapikachu, Atma gupta, Svyam gupta, Maharshibhi, Languli, Kandula, Chanda, Markati, Durabhigraha.According to the author of this text, Kapikachu is having madhura, tikta rasa, sheeta virya, and vata nashaka, vrishya, dusta vrana nasini. Atmagupta beeja taila is mentioned with having Madhura, Kashaya rasas, and Guru Ushna, Snigdha gunas.

Sodhala Nighantu (12thAD):

This Nighantu was composed by Sodhala in two parts named as Naamasangraha dealing with synonyms and Guna-Sagraha dealing with properties and actions. He has mentioned the Vrishya property of Kapikachu. In this text, Kapikachu is mentioned in Guduchyadi varga, with synonyms like Atmagupta, Svayamgupta, Ajaharsini, Languli, Kandura, Vyanda, Markati, Durabhigraha, Badari, Kapiroma phala, Dushparsha, Kacchura, Kharju, Romalu, Vanasukari. In this text, he also mentioned that it increases pitta, and its excess use, causes mada and vibhrama. In lakshmanadi varga he has mentioned one more drug with the name of Laghu kachu, and some of ayurvedic scholars consider this as *M.pruriens*.

Madanapala Nighantu (Madana Vinoda) (14th AD):

In this text, Kapikachu is mentioned in Abhayadi varga. Madanapala enumerated Kapikachu with its synonyms like Svayamgupta, Kandula, Duravagraha, Canda, Atmagupta, Languli, Markati, Praharshini. According to the author of this text Kapikachu is having Madhura rasa, Guruguna with actions like Vrishya and Brimhana. It is said as best for Vajikarana in this text.

Kaideva Nighantu (Pathya Pathya Vibhodaka) (15th AD):

In this text Kapikachu is described with the synonyms of Risyaprokta, Sukasimbhi, Kapiromphala, Rushabhi, Kapikacchu, Atmagupta, Kandura, Kandukari, Pravrushyani, Ajaha, Gupta, Svayam gupta, Markati, Kapiloma, Vrusya, Adhyanda, Vamani, Durabhigraha. In this text author mentioned that Kapikachu is having

Madhura Tikta rasa, Seeta virya and Vata pitta kapha and Rakta dosha nashaka. He has mentioned that, this drug has the properties similar to Masha and possesses Brimhana, Vrishya and Vrana shodhana qualities.

Bhavaprakasha Nighantu (16th AD):

This book is written by Bhavamishra, whose period is an important landmark in the history of Indian medicine. He stands at the junction of the medieval and modern periods. He revived the style of samhitas and contributed a good deal with various aspects of Ayurveda by adding new drugs and their indications. In this text Kapikachu is mentioned in guduchyadi varga, with the synonyms like Ajada, Atmagupta, Dushparsha, Kandura, Languli, Markati, Pravrshayani, Sukasimbi, Vrishya, Vyanga. According to this text Kapikachu is possessing Madhura, Tikta rasa, Guru guna, with the actions of tridosha samaka, vrishya. Vrishya property of Kapikachu beeja is accepted by this text.

Raja Nighantu (Nighantu Raja, Abhidaana chudammani) (17th AD):

In this text Kapikachu is mentioned in Guduchyadi varga and author (Vd Narahari) has described this drug with its synonyms like Vaanari, Languli, Atmagupta, Kandura, Markati, Sukasimbi, Syayam gupta, Kundali, Durabhigraha, Maharshabhi, Kundali, Karpi, Romphala, Gupta, Dussparsha, Kacchura, Jaya, Pravarushenya, Shukshimbhi, Badari, Guru, Aarshabhi, Shimbhi, Vaarahika, Tikshna, Romalu,Vanshukari, Kishoroma, Romavalli. Here in this text in respect of Kapikachu it is mentioned that this drug possess Madhura rasa, Virya Vardhaka, and useful in Vataroga, Kshaya, Shita pitta, Raktapitta and Vrana nashaka.

Rajavallabha Nighantu (18th AD):

This Nighantu is composed by Rajavallabha Vaidya and redacted by Narayana Dasa. This text deals with dietary articles with their properties and actions.This text is divided into six chapters.

Saligrama Nighantu (19th AD):

The author of this text is Lala Saligrama Vaidhya, son of Anandaroopa of Moradabad (UP, India). The text is divided into two sections and many modern drugs have been included in the text. In this text the author has followed other nighantus like

Nighantu ratnakara, Bhavaprakasha and Rajanighantu. In this text he has quoted synonyms, properties and actions of many drugs of other nighantus.

Priya Nighantu (By Acharya P.V.Sharma in 1983):

Acharya Priyavrat Sharma in his text mentioned Kapikachu in Pippalyadi varga with the synonyms of Atma gupta, Markati, Shuka simbhi. He has explained the guna karma of Kapikachu as having Madhura Tikta rasa, Guru guna, with Brimhana, Vata shamaka, Balavardhaka karmas.

2.2 Classification, Etymology, Synonyms, and Properties of Kapikachu in Ayurveda

2.2.1. Classification of Kapikachu

Table 2.11: Classification of Kapikachu

S.No.	Texts	Gana/Varga	References
1.	Caraka Samhita	Balya, Madhura Skanda,Pureesha Viranjaniya Vamanopaga	C.S.Su.4/7, C.S.Vi.8/139, C.S.Su.4/32
2.	Sushruta Samhita	Vidarigandhadi, Mudgadivarga, Kakolyadi, Vata Samsamana	S.S.Su.38/4, S.S.Su.46/36, S.S.Su.37/26
3.	Ashtanga Sangraha Ashtanga Hridaya	Vidaryadi,Durvadi Gana Vidaryadi	A.S.Su.16/2 A.H.Su.15/9
4.	Astanga Nighantu	Vidaaryadi	A.Ni.13.
5.	Dhanwantari Nighantu	Guducyadi	D.Ni.46/159
6.	Kaiyyadeva Nighantu	Ausadhi	K.Ni.154/11
7.	Bhavaprakasa Nighantu	Guducyadi	B.P.Ni. 57/131
8.	Sodhala Nighantu	Guduchyadi Varga Lakshmanadi Varga	S.Ni.171-172, 199-200. S.Ni.648
9.	Madana pala Nighantu	Abhyadi Varga	M.Ni.271.
10.	Priya Nighantu	Pippalyadi Varga	P.Ni.21/48-50

11.	Raja Nighantu	Guducyadi	R.Ni.50/52
12.	Adarsh Nighantu	Palashadi	A.Ni.453
13.	Dravyaguna Viyganam (Sharma, 1995)	Shukrajanaka	Page - 569

2.2.2. Synonyms of Kapikachu and their meaning

The authors of different Ayurvedic texts have mentioned several synonyms to the medicinal plants and minerals, while explaining their morphology, properties. In the same way for Kapikachu also different synonyms were given by scholars (Vaidya, 1998).

Atma gupta	:	Protects self with the help of its hair.
Ajada	:	Potent aphrodisiac
Durabhigraha	:	The fruits are difficult to handle.
Dushparsha	:	Produces irritable sensation on touching the fruit.
Kachura	:	Causes Itching.
Kapiromaphala	:	Pod hair resembles with monkey hair.
Markati	:	Hairy like monkeys
Kandura	:	Causes itching.
Languli	:	Legume's shape is like monkey's tail.
Pravishyana	:	Climber growing in rainy season.
Svayam Gupta	:	The plant is self protected by hairs on the fruit.
Vanshukari	:	Climber grows wildly.
Vrishya	:	Acts as aphrodisiac.
Vrishabhi	:	A potent aphrodisiac.
Vrushabeeja	:	The seeds are potent aphrodisiac.
Vyanga	:	Fruits are curved.
Kapikachu	:	Causes itching to monkeys also.
Suka simbhi	:	Pods/Seeds with hairs.

2.2.3 Etymology of Kapikachu

Literal meaning of the word "Kapikachu" is that this plant can cause itching to monkeys also (D.Ni).

2.3.4 Rasa Panchaka of Kapikachu

According to Ayurveda the knowledge of Rasa panchaka, that is Rasa, Guna, Virya, Vipaka, Prabhava are very essential to understand the activity and potency of any drug. Here the Rasapanchaka and dosha karma of kapikachu mentioned by different authors of Nighantus is given below in Table 2.12.

Table 2.12: Rasa Panchaka of Kapikachu

Rasa Pancaka		References							
		D.Ni.	M.Ni.	K.Ni.	B.Ni	R.Ni	S.Ni	P.Ni	API
RASA	Madhura	+	+	+	+	+		+	+
	Tikta	+			+		+	+	+
GUNA	Guru				+		+	+	+
	Snigdha				+		+		+
VIRYA	Sita	+				+			+
VIPAKA	Madhura						+		+
Dosha Karma-	Vatahara	+	+	+	+	+	+	+	+
	Kaphahara				+				+
	Pittahara	+	+	+	+	+	+		+

2.2.5. Pancha Bhoutika sanghatana of Kapikachu

Table 2.13: Pancha Bhoutika sanghatana of Kapikachu

S.No	**Rasa Panchaka**		**Pancha Mahabhuta constitution**
1	Rasa	Madhura Tikta	Prithvi+Aap Akasha + Vayu
2	Guna	Guru Snigdha	Prithvi+Aap Prithvi +Jala
3	Veerya	Seeta	Prithvi+Aap
4	Vipaka	Madhura	Prithvi+Aap

2.2.6. Guna karma of Kapikachu

Guna and karma are Ayurvedic pharmacodynamics of the plant. By knowing these, one can understand the specific properties and actions (dosha karma) of the drug. So that it can be better used in different diseases.

Rasa : Madhura Tikta.

Guna : Guru.

Virya : Ushna.

Vipaka : Madhura.

Karma (Dosha karma): Vata pittahara, Kapha hara (Bhava prakasha nighantu), Beeja is vatahara (Bhava prakasha)

Samanya karma : Vatahara, Vrishya, Brimhana, Balya, Mutrala, Stanya janana.

Rogagnata : Vata vyadhi, Atisara, Yoni roga, Vandhyata

2.2.7 Prayojyanga of Kapikachu (Useful part): Seed, Root, Hairs, Leaves (Anonymous, 1995; Sharma *etal.*, 2000; Anonymous, 2001; Sastry, 2014)

2.2.8 Prayoga (uses) of Kapikachu

Rakta Pitta, Dushtavrana (A.H, Chi, D.Ni / K.Ni.), Vajikara, Vata vyadhi (S.Ni), Atisara (S.S.Ut)

2.2.9. Yogas (Formulations) of Kapikachu

Kapikachu in different yogas mentioned by various samhitas

Table 2.14: Yogas (Formulations) of Kapikachu

S.NO	Name of the preparation	Indication	Reference
1	Bramha rasayana	Medho vridhi	C.S.Chi.1/1-58
2	Vrisya pupalika	For Vrishya action	C.S.Chi.2/3-15
3	Maha paisachika Ghrita	Fever, Insanity, Seizures	C.S.Chi.9/45-48
4	Swadamstradi Ghrita	Vata Pitta hara	C.S.Chi.11/44-47
5	Tryusanadya Ghrita	Kasa, Jwara, Gulmahara	C.S.Chi.18/39-42
6	Medicated Ghrita	Svasa, Kasa,Grahani	C.S.Chi.26/80-82
7	Jivantyadi Anuvasana Yamakam	Brimhana, Vatapitta nasaka, Bala, Sukra Agnivardhaka.	C.S.Si. 4/9-11
8	Vrishya yoga pupalika	Vrishya	C.S.Chi.2/2
9	Vrishya yog	Vrishyata	S.S.Chi. 26/30-35

10	Jeevantyadi Anuvaasana Yamakam	Vrishya, Deepana.	S.S.Chi. 37/23-26
11	Kapikachu churna	Pitta, kapha vidaghda drishti	S.S.Ut.17/7
12	Kapikachu Madhuyashti yoga	Vamana hara	S.S.Ut. 49/29
13	Kapikachu, Ikshurasa, Pippali Ghrita yoga	Mutraghata, Vandhyatwa	S.S.Ut.58/53-57
14	Kapikachu sidda Ghrita	Nasal drops to Garbhini	A.S.Su.29/11 and A.S.Su.7/31
15	Kapikachu mardana	Sanyasa/ Madatyaya	A.S.Chi.9/125
16	Nagabala ghrita	Kshata khaya, Pittarakta	A.S.Chi.5/69
17	Amrita taila	Vata vyadhi chikitsa, Agnideepana	A.S.Chi.23/72
18	Taila	Vatavyadhi chikitsa	A.S.Chi.23/86
19	Ghrita	Kshta Kshaya	A.S.Chi. 5/53
20	Kashaya preparation	Pittaja Chardi	A.S.Chi.8/19
21	Raja Yapana Basti	Aphordisiac / Vrishya	A.S.Ka.5/23
22	Yapana Bati	Vrishya / Vitality	A.S.Ka.5/34
23	Medicated Oil	Vata Pitta diseases related to sukra, Rajas.	A.S.Ka.5/49
24	Medicated Oil	Abhyanga	A.S.Ut.1/80
25	Maha kalyana Ghrita	Sannipaata vyadhis, Sthoulya	A.S.Ut.9/30
26	Maha paishachaka ghrita	Unmada, Apasmara, Sannipata Jawara	A.S.Ut.9/32
27	Medicated Oil	Paalitya Chikitsa	A.S.Ut.28/46
28	Maha mayura Ghrita	Cures Different Indriya vyadhis, Sirorogas.	A.S.Ut.28/73

29	Ksheeraa preparation	Vrishya , Visha	A.S.Ut.48/57
30	Saradi Ghrita	Vrishya	A.S.Ut.50/26,30
31	Beeja Churna with Ksheera	Vrishya	A.S.Ut.50/39,47.
32	Svayam Gupta Churna Yoga	Vrishya	A.S.Ut.50/58,60
33	Medicated Ghrita	Vrishya	A.S.Ut.50/62
34	Kapikachu molasidda Kheera and Ghee	Vrishya	A.S.Ut.50/64
35	Kapikachu sidha Poopalika with Ghrita	Vrishya	A.S.Ut.50/65, 68,69
36	Padalepa Yoga	Vrishya	A.S.Ut.50/106, 108
37	Medicated Ghee	Cough	A.H.Chi.3-38
38	Amrita prasa ghrita	Cough, Hiccup	A.H.Chi. 3/94-101
39	Svadamshtradi Ghrita	Vata pitta Hrid sula, Dysuria.	A.H.Chi.3/102-103
40	Agastya Rasayana (Hareetaki Rasayana)	Rasayana,	A.H.Chi 3/127-132
41	Vashishta (Hareetaki) Rasayana	Rasayana,	A.H.Chi.3/133
42	Jeevantyadi Kalpa (Vaata pitta hara kalpa)	Asthapana vasthi (Retention enema)	A.H.Ka.4/60
43	Medicated oil	Lepana for Karna pali Sandhana	A.H.Ut.40/17
44	Beeja churna ,masha flour with Ksheera	Vrishya	A.H.Ut.40/31
45	Kapikachu beeja churna, svadamshtra, ikshuras, satavari with milk	Vrishya	A.H.Ut.40/34
46	Kapikachu as diet	Vrishya	A.H.Su.6/22
47	Vidaryadi Ghrita	Vrishya	A.H.Ut.2/21,31,1/23

2.2.10 Dosage of Kapikachu:

Appropriate dosage of any drug is very important in the treatment of any disease. Physician should decide the dosage of any drug based on the patient condition age, strength, time, state of vitiated doshas and lastly disease.

Seed powder 3-6 g, Pod Hairs-125 mg, Root decoction: 20 – 50ml. (Anonymous, 2001; Nishteswar and Hemadri, 2013; Sastry, 2014)

2.3 Modern Literature of Kapikachu:

2.3.1 Vernacular names of Kapikachu (*Mucuna* sps) (Sharma *et al.*, 2000):

Hindi	:	Kounch
English	:	Cow-Hage or Cow – Itch.
Punjabi	:	Aalukusi.
Marathi	:	Khaajkuhili
Gujarati	:	Kavach.
Tamil	:	Punai kali.
Telugu	:	Doolagondi.
Kannada	:	Nasukunni.
Malayalam	:	Naikorana.
Sanskrit	:	Atmagupta, Risyaprokta, Khandura, Markati, Sukasimbi, Svayamgupta, Languli, Rishabhi, Adhyanda, Guptaphala, Svagupta, Ajadaphala.

2.3.2 Taxonimical classification of *Mucuna* sps.

Kingdom	:	Plantae
Division	:	Angiosperm
Class	:	Dicotyledons
Sub Class	:	Gamopetalae
Series	:	Bicarpellatae
Order	:	Leguminales
Family	:	Fabaceae
Sub Family	:	Faboideae
Genus	:	*Mucuna*

2.3.3 Genus Characters of *Mucuna* sps:

The plants of this genus are twining shrubs or herbs. Leaves pinnately 3-foliate, and stipules are deciduous, stipels minute. Flowers are large, purple or greenish, mostly fascicled on the tumid modes of racemes. These racemes are axillary or lateral on the branches or stems. Calyx tube is widely campanulate, the lowest tooth long, the lateral one short, and the two upperones are connate. Corolla much

excerted, standard about half the length of the wings and keel, auricled at base, Keel incurved. Stamens are diadelphous, anthers dimorphous, the longer basifixed, the shorter ovate or bearded. Ovary sessile, villous, 2 or many ovuled. Pods are usually covered with fine irritating pungent bristles. This genus comprises of 35 species and they are distributed among tropics, subtropics (Nadakarni, 1976).

2.3.4. Morphological Characters of *Mucuna pruriens* (L.) DC.

Annual hairy pea - like climber (twining herb) on shrubs and trees. Branches are tomentose when young. Stipule deciduous, petiole 10-15 cms, long, leaflets upto 16-19 cm, pubescent on both sides. Lateral leaflets very oblique and shorter than flowered raceme.

Flower, Calyx companulate, cleft half way down, clothed with shinning hairs. Corolla violet-purple in colour, keel exceeding standard and wings.

Pods sigmoid 5-8 cm long, 5-6 seeded, and irritating bristles are caducous. Pods turgid, turned upto end, longitudinally ribbed, covered with dense pale-brown bristles clothed with very pungently irritating sub-persistent pale brown or grey bristles in combage of falted moss of hairs. Hairy bristles 1 mm or upto 2.5 mm long, pointly, sharply 60-100 mm diam.

Seeds are matured, blackish white or black (mottled with black) abruptly hooked a tip and smooth which are obtained from ripe or matured pods.Flowering and fruiting time of *Mucuna* Sps is flowers in September– November and fruiting afterwards in January–April. (Cook, 1967; Naik, 1998; Singh and Karthikeyan, 2000)

Distribution: Plant is available throughout all tropical areas of the country from Himalayas, to Andaman Nicobar islands, and Srilanka, Burma plain regions in wild.

Although there are no varieties mentioned in classical literature of Ayurveda, some varieties are available in the market with the name of Kapikachu. They are

1. *Mucuna pruriens* (L) DC.: This is available all over India, and is quoted as Kapikachu by many research scholars / physicians and modern text books of Ayurveda. Hence, this plant seeds were used as field sample in this study.
2. *Mucuna cochinchinensis* (Lour.) A. Chev. (White seed): In most of markets of India especially in northern parts, this plant seeds are being used as Kapikachu. Hence, this is used as market sample in this study.

3. *Mucuna cochichinensis* (Lour.) A.Chev. (Black seed): This plant seed are used as Kapikachu in the southern parts of the country, especially in Kerala by Ayurvedic physicians. Hence, this plant also considered for study.

2.3.5. Pharmacological research works

- Seed powder of *Mucuna pruriens* showed hypotensive activity in dogs and spasmodic action in guinea pig (*in vitro* study) (Ramaswamy *et al*., 1979)
- The diet of *M. pruriens* seeds had produced hypoglycaemic effect in normal rats (Pant *et al*., 1968).
- Seed diet showed hypocholesterolemic effect in rats (Pant *et al*., 1968).

2.3.6. Substitutes / adulterants

It is observed that, *Mucuna utilis* Wall. Ex.Wight seeds are sold as Kapikachu in some markets (Garg, 1992).

2.3.7. Collection of Kapikachu pods / seeds

This is a tedious job and requires utmost care, skill. Any kind of negligence or direct touch with bristles of pods, leads to intense irritation (Urticarial rashes). Certain traditional methods are in practice by which plucking of dry pods that are hanging from trees, shrubs, and collection of seeds from those dry pods with the help of wet mud / clay.

2.4. Review of Pharmacognosy

Microscopic study of *M. pruriens* seed by Yelne and Sharma (1992), revealed that the hilum region is extended up to funicle composed of pitted sclereids with pointed and blunt ends. Sac like palisade layer containing xylem, phloem elements, stone cells. Vessels with spiral, reticulate, scalariform and pitted thickening are present. Outer most layer is composed of narrow, rectangular, radially elongated and compactly arranged palisade cells followed by bone shaped sclereids with inter cellular space. Cotyledon is composed of inner epidermal cells covered with thin cuticle followed by parenchymatous cells also consists of vascular strands and round to oval starch grains and also studied the physicochemical properties of the seed.

Similarly microscopic study of *M. pruriens* seed by Vijayambika *et al.,* (2011) shows at hilum region a wing shaped rim aril composed of thin walled parenchyma

cells. The palisade layer is narrow and composed of radially elongated cells followed by dumbel shaped hour glass cells. The tracheid elements are linear and compact with scalariform thickening which is surrounded by small, isodiametric, thin walled parenchymatous cells. Testa is conists of palisade layer followed by hour glass celss. Similarly T. S. of *M. cochinchinensis* seed shows hilum region with a pair of wing like rim aril with tapering ends pointing towards the micropyle followed by thick palisade layer. Testa composed of single layer of palisade cells followed by single layer of hour glass cells, oval to hexagonal loosely arranged parenchyma cells are present.

Kumaresapillai *et al.,* (2013) microscopic study of *M. cochinchinensis* seed revealed that thick raphe are present on either side of the hilar canal. It is composed of tracheid bar of spindle shaped cells with conical ends followed by two layered narrow, compact thick walled palisade cells. Seed coat is composed of narrow, compact, cylindrical or columnar (macrosclerides) cells of palisade layer covered with thin cuticle. It was followed by a bone shaped osteoscleride layer. Inner epidermal cells are small with prominent cuticle and filled with spherical starch grains with central hilum. Bone shaped osteosclerides, macrosclerides (columnar sclerides); large, spherical starch grains with central hilum.

2.5 Review of Phytochemical work

Human beings have relied on natural products as a resource of drugs for thousands of years. Plant based drugs have formed the basis of traditional medicine systems that have been used for centuries in many countries such as Egypt, China and India. At present plant-based drugs continue to play an essential role in health care. World Health Organization (WHO), observed that 80% of the population of the world rely mainly on traditional medicines for their primary health care. Currently at least 119 chemicals, derived from 90 plant species, can be considered as important drugs in one or more countries. Besides their direct medicinal application, natural products can also serve as pharmacophores for the design, synthesis or semi-synthesis of novel substances for medical uses. The discovery of natural products is also important as a means to further refine systems of plant classification. The most important of

bioactive compounds for any plants are alkaloids, flavonoids, tannins and phenolic compounds.

Phytochemistry:

The phytochemical screening of *Mucuna cochinchinensis* (Lour.) A. Chev. revealed the presence of alkaloids, phenols, flavonoids, amino acids, quinones, steroids and carbohydrate (Kumaresapillai *et al.*, 2013). The seed contains carbohydrate 55.8%, protein 27.5% and fat 3.6%. The fruits of *M. cochinchinensis* yield L-dopa (0.96%), which is an important drug for Parkinson's disease. The proximate composition and amino acid profile of *M. cochinchinensis* suggested that it could be a promising nutritional supplement (Ukachukwu and Obioha, 1997).

Seeds of velvet beans are known to produce the unusual nonprotein amino acid 3-(3,4-dihydroxyphenyl)-l-alanine (L-DOPA) (Lorenzetti *et al.*, 1998). It also contains glutathione, gallic acid and betasitosterol. It has unidentified bases like mucunine, Mucunadine, prurienine, prurieninine. Other bases isolated from the pods, seeds, leaves and roots include indole-3-alkylamines-N, N-dimethyltryptamine. Leaves also gave 6-methoxyharman. Serotonin is present only in pods (Khare, 2004). The seeds also contain oils including palmitic, stearic, oleic and linoleic acids (Mishra and Wagner, 2006).

GC-MS analysis showed the presence of phytochemicals like n-hexadecanoic acid (48.21%), Squalene (7.87%), Oleic acid (7.62%), ascorbic acid (3.80%) and Octadecanoic acid (6.21%) were present in the extract (Bhaskar *et al.*, 2011). The seed also contians two tetrahydroquinoline alkalodis namely (-) 3-methoxy-1, 1-dimethyl-6,7-dihydroxy-1,2,3.4-tetrahydroquinoline and (-) 3-methoxy-1,1-dimethyl-7,8-dihydroxy-1,2,3.4-tetrahydroquinoline (Mishra and Wagner, 2004). It also contains serotonin (5-hydorxy tryptamine, 5-HT), 5-hydorxy tryptophane (5-HTP), nicotine,N,N-dimethyl tryptamine (DMT), bufotenine, and 5-imethoxy-N,N-dimethyl tryptamine (5-MeODMT) 5-imethoxy-N,N-dimethyl tryptamine-noxide (5-MeO-DMT-n-oxide). The mature seeds of the plant contain about 3.1-6.1% L-DOPA, with trace amounts of serotonin, nicotine, Bufotenine, 5-MeO-DMT-n-oxide, and betacarboline. The leaves contain about 0.5% LDOPA, 0.006% DMT, 0.0025% 5-MeO-DMT and 0.003% DMT n-oxide (Kumar and Saha, 2013).

In addition to the low levels of sulfur-containing amino acids in *M. pruriens* seeds, the presence of anti-physiological and toxic factors may contribute to a decrease in their overall nutritional quality. These factors include polyphenols, trypsin inhibitors, phytate, cyanogenic glycosides, oligosaccharides, saponins, lectins, and alkaloids. Polyphenols (or tannins) are able to bind to proteins, thus lowering their digestibility. Phenolic compounds inhibit the activity of digestive as well as hydrolytic enzymes such as amylase, trypsin, chymotrypsin, and lipase. Recently, phenolics have been suggested to exhibit health related functional properties such as anti-carcinogenic, anti-viral, anti-microbial, anti-inflammatory, hypotensive, and anti-oxidant activities. Trypsin inhibitors belong to the group of proteinase inhibitors that include polypeptides or proteins that inhibit trypsin activity. Tannins exhibit weak interactions with trypsin, and thus also inhibit trypsin activity. Phytic acid [myoinositol-1,2,3,4,5,6-hexa (dihydrogen phosphate)] is a major component of all plant seeds, which can reduce the bioavailability of certain minerals such as zinc, calcium, magnesium, iron, and phosphorus, as well as trace minerals, via the formation of insoluble complexes at intestinal pH. Phytate-protein complexes may also result in the reduced solubility of proteins, which can affect the functional properties of proteins. Cyanogenic glycosides are plant toxins that upon hydrolysis, liberate hydrogen cyanide. The toxic effects of the free cyanide are well documented and affect a wide spectrum of organisms since their mode of action is inhibition of the cytochromes of the electron transport system (Laurena *et al*., 1994). Hydrogen cyanide (HCN) is known to cause both acute and chronic toxicity, but the HCN content of *M. pruriens* seeds is far below the lethal level. Janardhan *et al*. (2003) have investigated the concentration of oligosaccharides in *M. pruriens* seeds, and verbascose is reportedly the principal oligosaccharide therein (Siddhuraju *et al*., 2000). Fatty acid profiles reveal that lipids are a good source of the nutritionally essential linoleic and oleic acids. Linoleic acid is evidently the predominant fatty acid, followed by palmitic, oleic, and linolenic acids (Mohan and Janardhanan, 1995; Siddhuraju *et al*., 1996). The nutritional value of linoleic acid is due to its metabolism at tissue levels that produce the hormone-like prostaglandins. The activity of these prostaglandins includes lowering of blood pressure and constriction of smooth

muscle. Phytohemagglutinins (lectins) are substances possessing the ability to agglutinate human erythrocytes.

The major phenolic constituent of *M. pruriens* beans was found to be L-dopa (5%), along with minor amounts of methylated and non-methylated tetrahydroisoquinolines (0.25%) (Sidhuraju and Becker, 2001; Mishra and Wagner, 2004). However, in addition to L-dopa, 5-indole compounds, two of which were identified as tryptamine and 5-hydroxytryptamine, were also reported in *M. pruriens* seed extracts (Tripathi and Updhyay, 2001). Mucunine, Mucunadine, prurienine, and prurieninine are four alkaloids that have been isolated from such extracts (Mehta and Majumdar, 1994).

Antioxidant Activity:

Oxidative damages are either removed or repaired by host antioxidant defence mechanisms carried out by specific enzymes. Antioxidant properties are one of the most important claims for food ingredients, dietary supplements, cosmetics and anticancer natural products. The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytoconstituents. Because of multiple mechanism of actions and least toxic nature, plant based antioxidants are preferred rather than synthetic drugs. Antioxidants are widely used as food additives to provide protection against oxidative degradation of foods by free radicals. The bioactivity of flavonoids and related polyphenols appears to be mediated through a variety of mechanisms, though particular attention has been focused on their direct and indirect antioxidant actions. Antioxidants may protect cell constituents against oxidative damage and thereby limit the risk of various degenerative diseases associated to oxidative stress (Davide Grassi *et al.*, 2010). In plant cells, antioxidants help keep reactive oxygen species (ROS) at low concentrations, avoiding oxidative damage while allowing themselves play crucial functions in signal transduction (Manueli *et al.*, 2010). Antioxidants modulate the steady state concentrations of ROS, avoiding their potential cytotoxicity, while allowing them to function as signal molecules (Mittler *et al.*, 2004). Antioxidants have been found to possess anticancer, anticardiovascular, anti-inflammatory and other activities. So antioxidant is very much associated with anticancer activity. Enhanced antioxidant mechanism and

anticancer effect were reported for a flavone lupeol. Methanolic extract of *M.pruriens* exhibit significant antitumor and antioxidant effect in Erlich Ascites Carcinoma (EAC) bearing mice. The various parts of this plant contain total phenols which might have antioxidant activity. The similar findings were observed for this plant where free radical scavenging activity was evaluated via nitiric oxide scavenging method. The alcohol extract showed significant antioxidant activity which was comparable with standard ascorbate and total phenol content (Kumar and Muthu, 2010).

Free radicals that have one or more unpaired electrons are produced during normal and pathological cell metabolism. Reactive oxygen species (ROS) react readily with free radicals to become radicals themselves. Anti-oxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA strand breakage. Several substances from natural sources have been shown to contain anti-oxidants and are under study. Anti-oxidant compounds such as phenolic acids, polyphenols, and flavonoids, scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl, and thus inhibit oxidative mechanisms. Polyphenols are important phytochemicals due to their free radical scavenging and in vivo biological activities (Bravo *et al.*, 1998) the total polyphenolic content has been tested using Folin-Ciocalteau reagent. Flavonoids are simple phenolic compounds that have been reported to possess a wide spectrum of biochemical properties, including anti-oxidant, anti-mutagenic and anti-carcinogenic activity (Beta *et al*., 2005). The hydrogen donating ability of the methanol extract of *M. pruriens* was measured in the presence of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical. Extracts of whole *M. pruriens* plant (MEMP), which contains large amounts of phenolic compounds, exhibits high anti-oxidant and free radical scavenging activities (Kumar and Muthu, 2010). These in vitro assays indicate that this plant extract is a significant source of natural anti-oxidant, which may be useful in preventing various oxidative stresses. It has been reported (Ujowundu *et al*., 2010) that methanolic extracts of *M. pruriens* leaves have numerous biochemical and physiological activities, and contain pharmaceutically valuable compounds.

In vitro assays indicated that a whole plant of ethyl acetate and methanolic extract of *M. pruriens*, containing large amounts of phenolic compounds, exhibited high antioxidant and free radical scavenging activities. These plant extracts served as

a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. The various parts of this plant contain total phenols which might have antioxidant activity. The similar findings were observed for this plant where free radical scavenging activity was evaluated via nitiric oxide scavenging method. The alcohol extract showed significant antioxidant activity which was comparable with standard ascorbate and total phenol content (Kumar and Muthu, 2010; Kumar and Saha, 2013).

The antioxidant activity on *in vivo* models of lipid peroxidation concluded that the seed ethanolic extract of *Mucuna pruriens* has an antilipid peroxidation property which is mediated through the removal of super oxides and hydroxyl radicals (Tripathi and Upadhyay, 2001). Experiment on *in vitro* lipid peroxidation of *M. pruriens* seeds revealed the inhibition of ascorbate/$FeSO_4$ induced peroxidation by methanolic extract of *M. pruriens* which was monitored by the changes in optical density of the prepared concentrations (10-320 μg/ml). The inhibition increased with increase in concentration of the extract (Rajeshwar *et al.*, 2005).

2.6 Review of Pharmacology and Toxicology work

Antivenom Activity

The effectiveness of *M. pruriens* seeds aqueous extract (MPE) to neutralize the lethalities of snake venoms was investigated by in vitro neutralization and concluded as MPE effectively protect the animal models against lethality of *Naja sputatrix* venom and moderate protection against *Calloselasma rhodostoma* Venom. Indirect ELISA and immunoblotting studies showed that there were extensive cross-reactions between anti-MPE IgG and venoms from many different genera of poisonous snakes, suggesting the involvement of immunological neutralization in the protective effect of MPE pretreatment against snake venom poisoning. In vitro neutralization experiments also showed that the anti-MPE antibodies effectively neutralized the lethalities of Asiatic cobra (Naja) venoms, but were not very effective against other venoms tested (Tan *et al.*, 2009). Fung *et al.*, (2011) investigated antivenom activity of seeds where there was reduction in neuromuscular and cardiovascular depressant effects of *Naja sputatrix* venom in rats which was pretreated with MPE. The similar effects against *Calloselasma rhodostoma* Venom were observed. Where, MPE was given intra-peritoneal for 3 weeks. After 3 weeks,

Calloselasma rhodostoma venom was administered intra-venously and studied various pharmacology parameters like blood pressure, heart rate, respiratory rate and muscle twitch tension in rats. All pharmacological responses were found to be decreased in treated groups with respect to control group. Seed part showed strong antivenom activity which might be due to presence of higher amount of phytochemicals.

MPE were tested for their activity on various pharmacological effects like lethality, phospholipase activity, edema forming activity, fibrinolytic activity and haemorrhagic activity of cobra and krait venoms. About 0.16 and 0.19mg of MPE were able to completely neutralize the lethal activity of $2LD_{50}$ of cobra and krait venom, respectively, thus suggesting that MPE possess compounds, which inhibit the activity of cobra and krait venoms (Meenatchisundaram and Michael, 2010). According to Fung and Tan (2012), rats pretreated with MPE showed protective effect against the lethal and cardiovascular depressant effects of *Naja sputatrix* venoms by neutralization of the venom toxins.

The protective effects of MPE against histopathological changes induced by intravenous injection of *Naja sputatrix* (Malayan cobra) venom in rats pretreated with the MPE seed extract was also examined. Examination by light microscope revealed that the venom induced histopathological changes in heart and blood vessels in liver, but no effect on brain, lung, kidney and spleen. The induced changes were prevented by pretreatment of the rats with MPE. Finally it is suggested that MPE pretreatment protects rat heart and liver blood vessels against cobra venom induced damages (Fung *et al.*, 2009).

Hypoglycemic Activity:

The hypoglycemic activity of MPE was evaluated using streptozotocin induced diabetic, normal and glucose load condition rat models. The MPE at doses of 100 and 200mg/kg body wt. reduced oral glucose load from ~127 to 75 mg % after 2 hrs of oral administration. In another experiment there was reduction of blood glucose from ~250 to 90 mg % in streptozotocin diabetic rats after 21 days. The previous investigation suggested that the antidiabetic activity may be due to its dietary fiber content (Vadivel and Janardhan, 2000).

The hypoglycemic effect of MPE was investigated in normal, glucose load conditions and streptozotocin (STZ)-induced diabetic rats. In normal and STZ diabetic rats, the MPE (100 and 200 mg/kg body weight) significantly reduced the blood glucose levels 2 hrs after oral administration. It also significantly lowered the blood glucose in STZ diabetic rats after 21 days of daily oral administration of the extract. Thus, it was clearly depicted that MPE could be a source of hypoglycemic compounds (Bhaskar *et al.*, 2008).

Aphrodisiac Activity:

The quality of seminal changes due to psychological stress was assessed after treating the case with *M. pruriens* seed powder at 5g per day orally. For carrying out morphological and biochemical analysis, semen samples were collected twice, first before starting the treatment and second after 3 months of treatment. The results demonstrated the decreased sperm count and motility in subjects who were under psychological stress. Moreover, serum cortisol and seminal plasma lipid peroxide levels were also found elevated along with decreased seminal plasma glutathione (GSH) and ascorbic acid contents, reduced superoxide dismutase (SOD) and catalase activity. Treatment with *M. pruriens* significantly ameliorated psychological stress and seminal plasma lipid peroxide levels along with improved sperm count and motility. Treatment also restored the levels of SOD, catalase, GSH and ascorbic acid in seminal plasma of infertile men. *M. pruriens* not only reactivates the antioxidant defence system of infertile men but also helps in the management of stress and improves semen quality (Shukla *et al.*, 2007).

The effects of *M. urens* on the gonads of male guinea pigs were investigated and found to be the potential male antifertility agent even at a lower dosage of 70 mg/kg (Udoh and Ekpenyong, 2001). In another study “Kapikacchu Churna” effectively raised the sperm count. The results on sperm count found highly significant. It also showed good improvement in other seminal parameter like volume of semen, pH of semen, motility of sperms. It showed mild significant result in Non progressive sperm (NP) and not significant in Slow linear progress of sperm (SLP). It also significantly increased the sexual desire, penile rigidity, erection and duration of ejaculation with orgasm (Jadhav, 2013; Jadhav *et al.*, 2015).

For the study of impact of *M. pruriens* seeds on the metabolic profile of seminal plasma of infertile patients the proton nuclear magnetic resonance H^1NMR) spectroscopy was used. *M. pruriens* seed powder were administered to 180 infertile patients for a period of three months and for the comparison, a total of age-matched healthy men control (n=50) group was studied. By H^1NMR spectroscopy, lactate, citrate, glycerophosphocholine (GPC), choline, alanine, histidine, phenylalanine, tyrosine, uridine and glutamine were measured in seminal plasma and by using the standard laboratory methods and RIA, respectively, the degree of infertility and extent of hormonal imbalance induced by this milieu, separate sperm concentration, motility, lipid peroxide in seminal plasma and Lutienzing Hormone, Follicle Stimulating Hormone, Testosterone, and Prolactin hormone concentration in serum were examined and it was found that *M. pruriens* therapy rectifies the perturbed alanine, histidine, GPC, citrate and phenylalanine content in seminal plasma and improves the semen quality of post-treated infertile men with compared to pre-treated and along with this clinical variables in seminal plasma and blood serum were also improved over post therapy in infertile men. So it was concluded that *M. pruriens* seed powder rejuvenates the harmonic balance of male reproductive hormones in infertile men and reactivates the enzymatic activity of metabolic pathways and energy metabolism **(**Gupta *et al.*, 2011).

Dose and time-dependent effects of ethanolic extract of *M. pruriens* seed on sexual behaviour of normal male rats was studied (Sekar *et al.*, 2009). Effects of *M. pruriens* on semen profile and biochemical parameters in seminal plasma of infertile men were reported (Ahmad *et al.*, 2008).

M. pruriens and its major constituent L-DOPA recovered spermatogenic loss by combating ROS, loss of mitochondrial membrane potential and apoptosis (Singh *et al.*, 2013). The *M. pruriens* ethanolic extract increased mounting frequency, intromission frequency and ejaculation latency, and decreased the mounting latency, intromission latency, post-ejaculatory interval and interintromission interval. The potency test significantly increased erections, quick flips, long flips and total reflex. The ethanolic extracts of *M. pruriens* seed produced a significant and sustained increase in the sexual activity of normal male rats at a particular dose (200 mg/kg), when compared to the control (Suresh *et al.*, 2009). *Mucuna pruriens* improves male

fertility by its action on the hypothalamus-pituitary-gonadal axis. A study on treatment with *M. pruriens* significantly improved serum testosterone, luteinizing hormone, dopamine, adrenaline, and noradrenaline levels in infertile men and reduced levels of follicle stimulating hormone (FSH) and prolactine hormone (PRL). Sperm count and motility were significantly recovered in infertile men (Shukla *et al.*, 2009).

Antimicrobial Activity:

The methanolic extract of whole plant had antimicrobial properties against both Gram positive and Gram negative organisms (Rajeshwar *et al.*, 2005). This extract is mainly effective against *Escherichia coli, Salmonella typhi, Bacillus subtilis* and *Shigella dysenteriae.* The antimicrobial potency was evaluation by zone of inhibition (ZI) where *Escherichia coli* showed higher ZI (2.8cm) than *Bacillus subtilis* ZI (2.1cm) (Kumar *et al.*, 2009; Kavitha and Thangamani, 2014).

M. pruriens is also used for its antimicrobial properties against plant pathogenic bacteria and fungi. The methanolic extract showed high antibacterial activity against *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas marginalis, Pseudomonas acruginosa*, *Xanthomonas campestris* and high anti-fungal activity against *Curvularia lunata*, *Fusarium oxysporum*, *Pencillium expansum*, *Rhizoctonia solani*, *Tiarosporella phaseolina* and *Ustilago pomaydis* (Rayavarapu and Kaladhar, 2011). *Mucuna* monosperma has broad spectrum activity against Gram positive *Bacillus cereus*, *Staphylococcus* and Gram negative *Proteus vulgaris* (Manjunatha *et al.*, 2006).

Antiparkinson's Activity:

The clinical syndrome of Parkinsonism was identified in ancient India even before the period of Christ and was treated methodically. According to "Bhasava rajeeyam" the Parkinsonism was treated by the administration of powdered seed of *M. pruriens* containing 4 to 6% of levodopa (Ovallath and Deepa, 2013).

Clinical trials had been performed on *Mucuna*'s Parkinsonism action. Where 8 patients were treated with 15 and 30gm at *M. Pruriens* seed powder preparation for once in a week for 3 week. L-DOPA/Carbidopa was given in as combination dose of 200/50mg once which served as standard. The longer duration of action of seed

powder suggested that this might be having advantage for Parkinson disease for those patients having toxic effects to synthetic drugs (Katzenschlager *et al.*, 2004).

Hussian and Manyam, (1997) indicated that for the dose, *M. pruriens* showed twice the anti-Parkinsonian activity of synthetic L-DOPA. In a clinical study, (Nagashayana *et al.*, 2000) revealed the contribution of L-DOPA in the recovery of Parkinson's disease (PD) followed by Ayurveda medication. (Katzenschlager *et al.*, 2004) observed that 30g *Mucuna* seed powder preparation has considerable faster action in treating PD patients than conventional standard drugs, namely, Levodopa or Carbidopa and suggested that natural source of L-DOPA might possess advantages over conventional drugs in long term management of PD.

The efficacy and tolerability of HP-200, derived from *M. pruriens*, was examined in patients with Parkinson's disease. HP-200 developed from an alternative medicine source. A randomised, controlled, double blind crossover trial was carried out on 8 Parkinson‘s disease patients with a short duration L-dopa response and on period dyskinesias completed. Patients were challenged with single doses of 200/50 mg LD/CD, and 15 and 30 g of *Mucuna* preparation in randomised order at weekly intervals. On Compared with standard LD/CD, the 30 g *Mucuna* preparation led to a considerably faster onset of effect (34.6 v/s 68.5 min; p=0.021), reflected in shorter latencies to peak L-dopa plasma concentrations. Mean on time was 21.9% (37 min) longer with 30g *Mucuna* than with LD/CD (p=0.021); peak L-dopa plasma concentrations were 110% higher and the area under the plasma concentration vs time curve (area under curve) was 165.3% larger (p=0.012). No significant differences in dyskinesias or tolerability occurred. So, study proves that this natural source of L-dopa might possess advantages over conventional L-dopa preparations in the long term management of PD (Verma *et al.*, 2014).

This study evaluated the neuro restorative effect of *M. pruriens* cotyledon powder on the nigrostriatal tract of 6 hydroxydopamine 6-OHDA lesioned rats. *M. pruriens* cotyledon powder significantly increased the brain mitochondrial complex-I activity but did not affect the total monoamine oxidase activity (*in vitro*) as having Nicotine adenine dinucleotide (NADH) and coenzyme Q-10 in the cotyledon powder which are shown to have a therapeutic benefit in Parkinson‘s disease. Unlike synthetic levodopa treatment, *M. pruriens* cotyledon powder treatment significantly restored the

endogenous levodopa, dopamine, norepinephrine and serotonin content in the substantia nigra (Manyam *et al.*, 2004).

The extract of *Mucuna pruriens* used for antiparkinson's disease (MPE) is known to contain, among other components, 12.5% L-dihydroxyphenylalanine (L-dopa), as compared to the equivalent doses of L-dopa. An acute administration of MPE at a dose of 16 mg/kg (containing 2 mg/kg of L-dopa) consistently antagonized the deficit in latency of step initiation and adjusting step induced by a unilateral 6-hydroxydopamine lesion, whereas L-dopa was equally effective only at the doses of 6 mg/kg. At the same dosage, MPE significantly improved the placement of the forelimb in vibrissae-evoked forelimb placing, suggesting a significant antagonistic activity on both motor and sensory-motor deficits. The effects of MPE were moreover investigated by means of the turning behavior test and in the induction of abnormal involuntary movements (AIMs) after either acute or sub-chronic administration. MPE acutely induced a significantly higher contra lateral turning behavior than L-dopa (6 mg/kg) when administered at a dose of 48 mg/kg contains 6 mg/kg of L-dopa. On sub chronic administration, both MPE (48 mg/kg) and L-dopa (6 mg/kg) induced sensitization of contra lateral turning behavior; however, L-dopa alone induced a concomitant sensitization in AIMs suggesting that the dyskinetic potential of *M. pruriens* is lower than that of L-dopa. *M. pruriens* (48 mg/kg) was also effective in antagonizing tremulous jaw movements induced by tacrine, a validated test reproducing Parkinsonian tremor. Furthermore, *M. pruriens* induced no compartment preference in the place preference test, indicating the lack of components characterized by rewarding effects in the extract. Finally, in a sub-chronic mice model of 1- methyl- 4- phenyl- 1,2,3,6 tetrahydropyridine hydrochloride (MPTP) – induced dopamine neuron degeneration, MPE did not prove, capable of preventing either tyrosine hydroxylase decrease induced by MPTP or astroglial or microglial activation as assessed by means of glial fibrillary acidic protein (GFAP) and CD11b immunohistochemistry, supporting the absence of neuroprotective effects by *Mucuna pruriens.*

Also an another study proved the neuroprotective effects of *M. pruriens* in which the neurorestorative effect of *M. pruriens* cotyledon powder on the nigrostriatal tract of 6- hydroxyl dopamine (6-OHDA) lesioned rats was evaluated. The results

revealed that *M. pruriens* cotyledon powder significantly increased the brain mitochondrial complex-I activity but did not affect the total monoamine oxidase activity (*in vitro*) and unlike synthetic L-dopa treatment, *M. pruriens* cotyledon powder treatment significantly restored the endogenous L-dopa, dopamine, norepinephrine and serotonin content in the substantia nigra. Nicotine adenine dinucleotide (NADH) and coenzyme Q-10 that are shown to have a therapeutic benefit in Parkinson's disease were present in the *M. pruriens* cotyledon powder. Earlier studies showed that *M. pruriens* treatment controls the symptoms of Parkinson's disease. The additional finding of a neurorestorative benefit by *M. pruriens* cotyledon powder on the degenerating dopaminergic neurons in the substantia nigra may be due to increased complex-I activity and the presence of NADH and coenzyme Q-10 (Manyam *et al.*, 2004).

Anti-Depressant activity:

This involves the study of anti-depressant-like effects of *M. pruriens* in acute and chronic model of depression. Psycho-pharmacological investigation involved treatment (14 days) of *Mucuna* in forced swim test (FST), tail suspension test (TST) in mice and olfactory bulbectomy in rats, respectively. The investigations results show the initial anti-depressant-like effect of *Mucuna* (10-20 mg/kg i.p.) or (10 mg/kg) significantly enhanced the anti-depressant action of fluoxetine and bupropion in mice FST and TST respectively. Potentiation of 5-Hydroxytryptophan induced head twitches response (in mice) and reversal of reserpine induced hypothermia (rats) were observed at same dose level. Further, the behavioural anomalies exhibited by olfactory bulbectomised rats (OBX) were attenuated by chronic *Mucuna* treatment as observed in open field (Patil *et al.*, 2010).

Anti-Inflammatory activity:

This involved evaluating the effect of the methanolic extract of *M. pruriens* seed in mice with respect to delayed hypersensitivity reaction (DTR), primary and secondary antibody response and In vivo inflammatory leukocyte mobilization. The extract caused elevation of secondary SRBCs-specific antibody titre with antibody response being significantly ($p<0.05$) increased at 250 and 500 mg/kg when compared with control. The results showed that the extract at 250 and 500 mg/kg produced

significant ($p<0.05$) inhibition of DTR in mice by 33.33% and 28.89%, respectively and hence, can probably influenced immune response in mice (Eze and Ndukwe, 2012; Natarajan *et al.*, 2012).

Antitumour effect:

The antitumour effect and antioxidant role of methanolic extract of *Mucuna pruriens* seed against Erlich Acites Carcinoma (EAC) bearing Swiss albino mice were studied. The effect of methanolic extract of *M. pruriens* on tumor growth and host's survival time was studied by the following parameters; tumor volume, packed cell volume viable and non viable cell count and life span of the host. Extract was administered at 125 and 250 mg/kg body weight once daily for 14 days, starting after 24 h of tumor inoculation. Decrease in tumor volume, packed cell volume and viable cell count were observed in extract treated animals when compared to EAC treated animals. Treatment with extract at a dose of 125 and 250 mg/kg increased the mean survival time to 29.5± 0.55 and 34±0.2 days respectively. The extract also decreased the body weight of the EAC tumor bearing mice. There was a significant decrease in WBC count and increase in RBC counts in extract treated animals when compared to EAC treated animals. The study was also extended to estimate the liver biochemical parameters such as LPO, GSH and antioxidant enzymes like SOD, catalase etc. Treatment with extract decreased the levels of lipid peroxidation and increased the levels of glutathione, superoxide dismutase and catalase. The results suggest that the methanolic extract of *M. pruriens* seeds exhibits significant antitumor and antioxidant effects in EAC bearing mice (Rajeshwar *et al.*, 2005).

Antidiabetic effect:

Many reports have been established relating to antidiabetic property of *Mucuna*. The hypoglycemic activity of *M. pruriens* ethanolic extract in alloxan induced rats and streptozotocin induced mice produced the maximum activity at 6^{th} week in 200 mg/kg/day dose (Rathi *et al*, 2002). A comparative study of the hypoglycemic effect of aqueous extract of the seeds of *M. pruriens* between normal, glucose load conditions and streptozotocin induced diabetic rats were analyzed. The results showed that in normal rats, the aqueous extract of the seeds of *M. pruriens* (100 and 200 mg/kg body weight) significantly ($p<0.001$) reduced the blood glucose

levels after an oral glucose load from 127.5±3.2 to 75.6±4.8 mg % 2 h after oral administration of MPE seed extract. It also significantly lowered the blood glucose in streptozotocin induced diabetic rats from 240.5±7.2 to 90.6±5.6 mg % after 21 days of treatment ($p<0.001$). Thus, the study concluded that *M. pruriens* has an antihyperglycemic action and it could be a source of hypoglycemic compounds (Bhaskar *et al.*, 2008). Comparative evaluation of hypoglycemic activity of some ethanolic extracts of 24 Indian medicinal plants in alloxan induced diabetes condition was done and the plants were positioned according to the significant blood glucose lowering activities in decreasing order, *Mucuna prurita* was at 17th place (Kar *et al.*, 2003).

Antiprotozal effect:

Methanolic extract of leaves of *Mucuna pruriens* has the potency to eradicate *Lichthyophtirius multifilis* infection (90%) in gold fish after treatment in baths of plant extracts at 200 mg/ liter and parasite induced fish mortality was reduced significantly. The *in vitro* studies shows 100% mortality of parasite tested in 150 mg/liter of *M. pruriens* extract (Ekanem *et al.*, 2004).

Toxicology:

This plant has no such toxicological consensus but has several limitations. It is toxic to human and animal due to the presence of L-DOPA and tryptamines. Trypsin enzyme activity has been inhibited by seed. The undried leaves have hairy substances which has itching capacity (tropicalforages.info).

The toxic principle in *Mucuna* seed is held to be L-Dopa, 3-(3,4-dihydroxyphenyl alanine) a compound chiefly used in treating the symptoms of Parkinson's disease. In addition to gastrointestinal disturbances — notably, nausea, vomiting, and anorexia — the most serious effects are reported to be aggression, paranoid delusions, hallucinations, delirium, severe depression, with or without suicidal behavior, and unmasking of dementia. Psychotic reactions are more likely in patients with postencephalitic Parkinsonism or a history of mental disorders (Reynolds, 1989).

In 1989, in Nampula, Mozambique, an outbreak of more than 200 cases of acute toxic psychosis was attributed to consumption of the seeds of *M. pruriens*

(Infante, 1990). The seeds are usually detoxified by repeated boiling in water, which is discarded before further processing of the seed, but because of drought the people drank this water instead. *Mucuna* sps. have also been reported to contain, in addition to L-Dopa, antinutritional factors, such as phenols and tannins, and to possess trypsin inhibiting and haemagglutonating activities (Rajaram and Janardhanan, 1991). The presence of nicotine, physostigmine, and serotonin in *Mucuna* was also reported, it was also claimed that this last compound, the important neurotransmitter also known as 5-hydroxytryptamine (5-HT), is present in the golden-yellow trichomes of pods of *M. pruriens*. In addition, bufotenine, choline, N,N-dimethyltryptamine (DMT), DMT-Nb-oxide, 5-methoxy-DMT as well as two unidentified 5-oxy-indole-3-alkylamines, an unidentified indole-3-alkylamine, and an unidentified ß-carboline, were isolated from a mixture of the pods, seeds, leaves, and roots of *M. pruriens* using thin-layer chromatography (Lorenzetti *et al.*,1998).

2.7. Review of DNA fingerprinting by RAPD technique of Kapikachu

The Polymorphic chain reaction (PCR) based Randomly Amplified Polymorphic DNA (RAPD) has been a convenient and handy technique to analyze the genetic variation (Bhat *et al*., 1999). It has been extensively used for molecular fingerprinting and population diversity analysis (Albert *et al*., 1997, Saini *et al*., 2010) and successfully employed in the construction of linkage maps. Availability of reliable polymorphic markers, often limits the accurate estimation of genetic variation among individuals or different populations. The extent of genetic variation between geographical populations depends upon several factors, including gene flow between populations (Baker and Stebbins, 1965; Barrett, 2015).

In vitro clonal propagation of *Mucuna pruriens* var. *utilis* was carried out by Sathyanarayana *et al*., (2006). They confirmed the genetic fidelity and stability through RAPD markers employing 15 operon primers. Genetic diversity of *Mucuna* sp was estimated in a collection of 64 *Mucuna* sp by Capo-chichi *et al*., (2003) from different eco-geographical regions using amplified fragment length polymorphism (AFLP). The level of genetic variability within the AFLP analysis suggested that it is reliable, efficient and effective marker technology for delineating genetic diversity.

PCR based DNA fingerprinting method (RAPD) is used to identify the highest yielding trichome-less elite strain of *Mucuna* by Dhawan *et al*. (2011). They obtained

the genetic similarity index matrix through multivariant analysis using Nei and Li's coefficient. The similarity coefficients were used to generate a tree for cluster analysis using the UPGMA method. Genetic diversity of velvet bean, *Mucuna pruriens* (L.) DC. was assessed by Kalidass and Mohan (2010) from Western Ghats, Tamil Nadu, India.

RAPD techniques have been found to be more useful and accurate for the determination of both inter-specific and intra specific variations in plants. In particular, RAPD markers have been successfully employed for the determination of intra-specific genetic diversity in several species (Wolfe and Liston, 1998). In contrast, only few reports were available on the determination of inter-specific diversity in plants using the RAPD technique, which yield information about the phylogenetic of the plant (Singh *et al*., 2006; Carlos *et al*., 2006; Ranade *et al*., 2006; Lim *et al*., 2007; Singh *et al*., 2007; Parab *et al*., 2008; Pawel *et al*., 2009; Dhanasekar *et al*., 2010).

श्लोक

कैयदेवनिघण्टु

ऋष्यप्रोक्ता शूकशिम्बी कपिरोमफलार्षिभिः |
कपिकच्छुरात्मगुप्ता कण्डुरा प्रावृषायणी ||६०६||
कण्डूकर्यजहा गुप्ता स्वयङ्गुप्ता च मर्कटी |
कपिलोमा वृषाध्यण्डा वामनी दुरभिग्रहा ||६०७||
कपिकच्छूः स्वादुतिक्ता वातपित्तकफास्रजित् |
शीतलं बृंहणं वृष्यं माषतुल्यं तयोः फलम् ||६०८||
कपिकच्छूफलं वृष्यं शीतं स्वादुरसं गुरु|
रक्तपित्तानिलहरं दुष्टव्रणविशोधनम् ||६०९|| **कै. नि. १ / कपिकच्छू (६०६-६०९)**

भावप्रकाशनिघण्टु

कपिकच्छूरात्मगुप्ता वृष्याप्रोक्ता च मर्कटी ।
अजडा कण्डुरा व्यङ्गा दुःस्पर्शा प्रावृषायणी ।
लाङ्गली भूकारीम्बी च सैव प्रोक्ता महर्षिभिः।
कपिकच्छूर्भृशं वृष्या मधुरा बृंहणी गुरूः ।
तित्का वातहरी बल्या कफपित्तास्ननाशीनी ॥
तद्वीजं वातरामनं स्मृतं वाजीकरं परम्॥ (भा.प्र.)
यस्माच्छुक्रस्य वृद्धिः स्याच्छुक्रलं हि तदुच्यते |
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लाङ्गूली कुण्डली चण्डा मर्कटी दुरभिग्रहा ॥
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Chapter 3: Materials and Methods

A. Pharmacognostical study

3.1 Collection of plant material

Fresh, healthy seeds of *Mucuna pruriens* (L.) DC. which were free of diseases were collected from NRIBAS, garden and seeds of *Mucuna cochinchinensis* (Lour.) Chev. (White) were purchased from local market of Pune, and from Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra respectively. Whereas, seeds of *Mucuna cochinchinensis* (Lour.) Chev. (Black) were purchased from local market of Calicut, Kerala.

3.2 Authentication of plant material

Collected crude material (seeds of 3 *Mucuna* sps) were identified and authenticated by Agharkar Research Institute, Pune and deposited with voucher number, S-162 *Mucuna pruriens* (L.) DC., Voucher number S-161, for *Mucuna cochinchinensis* (Lour.) A. Chev. (White seed) and voucher number S-159 for *Mucuna cochinchinensis* (Lour.) A. Chev. (Black seed).

3.3 Pharmacognostic studies

Macro and microscopic analysis of *Mucuna* sps seeds was performed in accordance with standard laboratory practices. Dinolite camera and binocular microscope (Biolux) were used to take photomicrographs of seed sections and powder study of *Mucuna* sps. Water, Iodine, Phloroglucinol and Hydrochloric acid (HCl) were used to prepare slides of lignified tissues. Glycerin was used for mounting the sections. Pharmacognostic analysis of seeds was done by following the methods mentioned in Ayurvedic Pharmacopoeia of India, W.H.O. guide lines and Johansen (1940).

3.3.1 Macroscopic and organoleptic characters

Plants and seeds of *Mucuna* species were photographed. The morphological characters and the organoleptic characters like color, texture, taste, odour of seed powder were observed and documented as identification markers of individual species.

3.3.2 Microscopic study

Freehand sections of seeds were taken with the help of platinum razor blades. Sections were treated with phloroglucinol, followed by hydrochloric acid (HCl), Iodine, and Sudan red‑III for identification of various cellular details. Microphotographs were captured with the help of dinolite camera which was attached to the microscope.

B. Phytochemistry of *Mucuna* sps

3.4 Physicochemical analysis

3.4.1 Determination of Foreign Matter

Foreign matter is the one which is not coming from the original plant source as defined, and can be a contamination by insects, animals, moulds or by any other sources like parts of the organ or organs from which the drug is derived.

a. 100 g of sample was weighed.

b. The sample was spread on a white tile or glass plate uniformly without overlapping.

c. Inspected the sample with naked eyes and by means of a lens (5x or above).

d. The foreign matter (mentioned above) was separated manually.

e. After complete separation, the foreign matter was weighed and determined percent weight by weight (% w/w) present in the sample.

Percentage of foreign matter was calculated using the following formula. For which gross weight of the sample was noted.

% of Foreign matter = (Gross weight of the sample – Weight of sample after removing foreign matter) /100x 100.

3.4.2 Determination of total Ash Value

This is used to determine the quality and purity of the crude drug. Ash contains inorganic materials like Phosphates, carbonates, and silicates of sodium, potassium, magnesium, calcium etc.

a. Silica crucible was initially weighed.

b. 2-3 g of powder drug was taken in crucible and kept it in the furnace at a temperature not exceeding 450ºC until it got free from carbon. The crucible was then cooled in a desiccator, and the ash weight was noted. Percentage of total ash was calculated with reference to the air dried sample of the crude drug.

Calculations were done by using the following formula: Total Ash = (z-x) / y X100.

(x: weight of empty crucible, y: weight of sample, z: weight of crucible after ignition).

3.4.3 Determination of Acid- Insoluble Ash value

Sometimes the presence of inorganic materials like calcium oxalate, silica, and carbonate in the crude drug affects the total ash value. Hence such materials were removed by treating with Hydrochloric acid and the acid insoluble ash value is measured. Acid insoluble ash value is always less than total ash value.

The ash obtained from the total ash content, was boiled for 5 minutes with 25 ml of dilute hydrochloric acid, the insoluble matter obtained was collected on an ashless filter paper (Whatmann No. 40, 90 mm diameter), which was washed with hot water and ignited the crucible in the flame. Then crucible is cooled, and weighed. The filter paper and residue was put together into the crucible, heated gently until vapours stops and heated further till carbon disappears. The crucible was cooled in a desiccator. The percentage of acid-insoluble ash was calculated using the formula**:**

Acid insoluble ash = (z-x)/y X100. (where: x: weight of empty crucible, y : weight of sample, z: weight of crucible after ignition).

3.4.4 Determination of Water soluble Ash value

This is measured in a similar way to acid insoluble ash, using 25ml water, in place of dilute hydrochloric acid and calculated by using the following formula.

Water soluble ash = (z-x) / y X 100. [Where, x: weight of empty crucible, y: weight of sample, z: weight of crucible after ignition].

3.4.5 Determination of Moisture (Loss on Drying)

The loss on drying was determined by taking 2 g of powder (sample) in a dried Petri-dish and dried it in an oven at 105ºC constantly. The weight after drying was noted and percentage loss on drying was calculated on the basis of air-dried sample using the following formula.

$$\text{Percentage of LOD} = \frac{\text{Loss on drying}}{\text{Weight of sample}} \times 100\% \text{ w/w}$$

3.4.6 Determination of pH Values

10 g seed powder was weighed, 100 ml distilled water was added to it so as to get 10% solution. It was then mixed continuously with the help of clean and dry glass rod for about 45 minutes till it became a homogenous mixture. It was then filtered through filter paper so as to remove the insoluble components. The pH meter was calibrated using standard buffer solution of pH 7 and pH 9 and then pH value was measured on pH meter (Metler, Toledo).

3.4.7 Determination of swelling factor

The measurement of swelling factor gives an idea about the mucilage content of the seed, hence it is useful in the evaluation of crude drugs containing mucilage. One gram seeds were taken in 25 ml stopper cylinder and 25 ml water was added. This was then incubated at room temperature for 24 hrs with occasional shaking. Final volume of leftout water was measured.

3.4.8 Determination of Extractive Values

5 g seed powder of all the study samples were macerated separately with 100 ml solvent (Alcohol, water, Acetone, Methanol, Petroleun Ether, Ethyl Acetate, Benzene, Chloroform, Carbon Tetra Chloride, and water + alcohol separately) in a closed flask for 24 hours. The flask was intermittently shaken during the first 6 hours and allowed to settle without any movement / disturbance for 18 hours. The extract was filtered with precaution avoiding solvent loss and 25 ml of the filtrate was evaporated to dryness in a dried, weighed, tared flat bottom evaporating dish and dried at 105°C to a constant weight. From the weight of the residue obtained, the percentage of alcohol soluble extractive was calculated in accordance to the air-dried drug and then percentage of alcohol soluble extract was calculated using the following formula.

Percentage of alcohol soluble extract = (Weight of residue x100x100/ weight of sample x25) % w/w.

3.4.9 Fluorescence analysis

Extracts obtained from different solvents of three *Mucuna* sps separately, such as distilled water, acetone, benzene, chloroform, carbon tetra chloride, Absolute

alcohol, ethyl acetate, methanol and petroleum ether were observed under ultra violet (UV) light at 254 nm and 366 nm wavelengths and under day light.

3.4.10 Determination of Dimensional parameters (Length, Breadth, and Width) of seeds of *Mucuna species*

Measurements for length, breadth and width of 25 seeds of all three *Mucuna* species under study were noted (individual seed). The average of them was calculated to determine the dimensions of seed of each species.

3.4.11 Determination of weight variation of seeds of *Mucuna* species

100 seeds were weighed separately for each species and the average of them was taken as ideal weight of the seed of individual *Mucuna* species.

3.4.12 Determination of Specific Gravity of *Mucuna* species with different solvents

The weight per milliliter of a liquid is the weight in gram of 1 ml of a liquid when weighed at 25°C. The specific gravity was determined using Pycnometer and standard procedure as mentioned in Kokate *et al.*, (1996).

3.5 Qualitative analysis

The methods used to isolate active substance (components) are termed as extractive method. Crude extracts obtained from such extraction can be qualitatively tested to ascertain the presence of different components. Qualitative tests are used to detect the presence of functional groups, which play very important role in the expression of biological activity. Using methanol and water soluble extracts of seed powder, qualitative and quantitative tests were carried out. These tests indicate the types of phyto-constituents present in the sample.

3.5.1 Test for carbohydrates

Molish's test (General test): To 2-3 ml extract, few drops of *Molisch reagent* was added, mixed well and conc. H_2SO_4 was added from the sides of the test tube, violet ring formation at the junction of two liquids confirms the presence of carbohydrates (Krishnaveni *et al.*, 1984).

3.5.2 Test for reducing sugar

(a) *Fehling's test*: 1 ml Fehling's A and B solutions were mixed and mixture was boiled for 1 min and equal volume of sample solution was added and then again boiled on water bath for 5-10 min. Appearance of yellow to brick red precipitate showed the presence of reducing sugar.

(b) *Benedict's test*: Equal volume of Benedict's reagent and sample solution were added in test tube and then it was boiled on water bath for 5 min. Appearance of green, yellow or red colour (depending on amount of reducing sugar present in sample solution) confirmed the presence of reducing sugar.

3.5.3 Test for pentose sugar:

(a) *Aniline acetate test*: Sample solution was boiled, and filter paper presoaked in aniline acetate turned to pink colour when exposed to the vapours, which confirms the test positive.

(b) Equal amount of sample solution and HCl was mixed, heated, and a crystal of phloroglucinol was added which turns the solution into red colour and confirmed the presence of pentose sugar.

3.5.4 Test for Hexose sugar

(a) *Tollen's Phloroglucinol test* for galactose: 2.5 ml of concentrated HCl and 4 ml of 0.5 % phloroglucinol were mixed with 1-2 ml test solution and warmed. Appearance of yellow to red colour indicates the test positive.

(b) *Cobalt-Chloride test*: 3 ml of sample solution was mixed with 2 ml of cobalt chloride, boiled and then cooled. Afterwards, few drops of NaOH solution added. Appearance of Greenish blue colour confirms the glucose presence. Similarly, presence of fructose was confirmed by appearance of purplish colour. When upper layer and lower layer showed different colours (greenish blue and purplish respectively) confirmed the presence of glucose and fructose.

3.5.5 Test for protein

(a) *Biuret test* (General test): Presence of proteins was determined by Biuret test. 3 ml of sample solution was added to 4% NaOH and to this resultant solution, few drops of 1% $CuSO_4$ solution was added. Appearance of violet - pink colour indicates the presence of proteins in the sample solution (Lowry *et al.*, 1951).

(b) *Million's test*: To carry out Million's test, 3 ml of sample solution was mixed with 5 ml Million's reagent. Appearance of white precipitate after warming this solution appearance of red color solution confirmed the test is positive for proteins.

(c) *Xanthoprotein test*: To 3 ml sample solution 1 ml conc. H_2SO_4 was added, which resulted in forming the white precipitate. On boiling, precipitate turned into yellow and the on adding NH_4OH, precipitate changed into orange colour making test positive.

(d) *Test for proteins containing Sulphur*: 5 ml sample solution was mixed with 2 ml of 40% NaOH and 2 drops of 10% lead acetate was added. On boiling this solution, turned into black to brownish confirms the presence of proteins containing sulphur.

(e) *Precipitation test*: The sample solution giving white colloidal precipitate with Absolute alcohol, 5% $Hgcl_2$, 5% $CuSO_4$ solution, 5% Lead acetate, 5% Ammonium sulphate confirming the presence of proteins in the test solution.

3.5.6 Test for Amino acid:

(a) *Ninhydrin test* (General test): *Ninhydrin test* was conducted for amino acids in general and presence of cysteine was checked by adding 40% NaOH and 10% lead acetate solution to sample solution. Appearance of black lead sulphate precipitate after boiling confirmed the test (Sadasivam and Manickam, 1996).

(b) *Test for cysteine*: To 5 ml of sample solution few drops of 40% NaOH and 10% lead acetate solution was added and boiled. Appearance of black precipitate of lead sulphate was formed confirming the presence of Cysteine.

3.5.7 Test for Steroids

(a) *Liebermann-Burchard Test*: It was conducted for steroids. While performing test to 2 ml sample solution with chloroform, 1-2 ml of acetic anhydride, and few drops of conc. H_2SO_4 was added from the side of test tube. Presence of Steroids was proved by appearance of reddish brown coloured ring at the junction of two layers (Wall *et al*., 1954). (b) *Salkowski reaction*: To 2 ml of sample solution, 2 ml of chloroform and 2 ml of conc. H_2SO_4 were added. Then tube was Shook well, Chloroform layer was appeared red and acid layer shows greenish yellow fluorescence confirming the test positive.

3.5.8 Test for Glycosides

To 2 ml sample solution, few drops of glacial acetic acid, 5% $FeCl_3$ and conc. H_2SO_4 were added. Appearance of reddish brown colour at the junction of two liquid layers and upper layer appears bluish green indicating the presence of glycosides (Trease and Evans, 1989; Harborne, 1998).

3.5.9 Test for Cardiac glycosides

(a) *Legal test*: To aqueous or alcoholic sample extract, a few drops of pyridine were added. A drop of 2% sodium nitroprusside and a drop of 20% sodium solution were added. Formation of deep red colour indicated the presence of Cardiac glycoside.

(b) *Keller-Killiani test*: To 2 ml of sample extract, few drops of glacial acetic acid, one drop of 5% $FeCl_3$ and H_2SO_4 were added. Appearance of green-blue colour in the upper acetic layer shows the test positive.

3.5.10 Test for Anthroquinone glycosides

(a) *Borntrager's Test*: To 3 ml of sample extract, few drops of dil. H_2SO_4 were added. This solution was boiled and filtered. To this cold filtrate, equal volume of benzene or chloroform was added and shook well. The organic solvent was separated and Ammonia solution was added. Ammonia layer turned into pink to red when test was positive.

(b) *Modified Borntrager's test for C-glycosides*: To 5 ml of sample extract, 5 ml 5% $FeCl_3$ and 5 ml dil. HCl were added and heated it for 5 min in boiling water bath. Cooled and on adding benzene or any organic solvent and shook well. After separating organic layer and adding equal volume of Ammonia solution. Ammonia layer showed pinkish red colour when test was positive.

3.5.11 Test for Saponins

Foam and *lead acetate tests* were carried out for indicating the presence of saponins. The layer of foam formation on vigorous shaking of sample extract and distilled water solution confirmed the presence of saponins in foam test and formation of white precipitate on the addition of lead acetate indicated the presence of saponins (Siddiqui and Ali, 1997; Tiwari *et al.*, 2011).

3.5.12 Test for Cyanogenetic glycosides

(a) *Grignard reaction or sodium picrate test*: 200 mg of plant material was placed in a conical flask and moistened it with few drops of water. To moistened sample a piece of picric acid paper with 5% aqueous sodium carbonate solution, was suspended by means of cork in the neck of the flask. The change in colour was observed. Hydrogen cyanide liberated from cyanogenetic glycosides by the enzyme activity is reacted with sodium picrate to form the reddish purple sodium isopurpurate confirmed the presence of cynogenetic glycosides.

(b) To dry plant powder or extract, few drops of 3% aqueous mercurious nitrate solution was added. Formation of metallic mercury denotes test as positive.

3.5.13 Test for coumarine glycosides

Coumarin glycosides have aromatic odour. Alcoholic extract sample was made alkaline, and observed for colour change (blue or green fluorescence colour shows presence of coumarine glycosides).

3.5.14 Test for Flavonoid

(a) *Shinoda Test*: An alcoholic extract of sample was treated with magnesium (dust) and conc. HCl. The appearance of pink tomato colour indicated the presence of flavonoids.

(b) To small quantity of residue, lead acetate solution was added then yellow coloured precipitate was formed denoting the presence of flavonoids.

(c) Addition of sodium hydroxide to the residue showed yellow colour, which decolourises on addition of acid confirming the presence of flavonoids.

3.5.15 Test for Alkaloids

The aqueous and alcoholic extract was evaporated separately. To this residue, dil. HCl was added, which was then filtered. Tests were performed with filtrate.

(a) *Dragendroff's test*: To 2-3 ml of filtrate few drops of *Dragendroff's reagent* was added, formation of Orange brown precipitate showed the test positive.

(b) *Mayer's test*: To 2-3 ml filtrate few drops of *Mayer's reagent* formed precipitate confirming the presence of alkaloids.

(c) *Wagner's test***:** To 2-3 ml of sample's filtrate few drops of *Wagner's reagent* was added which resulted in the formation of reddish brown precipitate, showing the test positive.

3.5.16 Test for Tannins

(a) To an aqueous solution of dried sample material, 5% lead acetate solution was added, which lead to appearance of white precipitate indicating the presence of tannin.
(b) To an aqueous solution of dried plant material add 1% solution of gelatin was added, appearance of white precipitate indicated the presence of tannin.

3.5.17 Test for phenols

(a) Small amount of methanol extract was treated with neutral $FeCl_3$. Formation of blue or green colour indicated the presence of phenol.
(b) *Folin-ciocaltaue reagent*: The sample solution treated with a little residue of ammonia solution, appearance of blue or blue gray colour indicating the presence of phenols.
(c) *Indophenol reaction*: The extract of sample material was treated with a few drops of conc. H_2SO_4, a few crystals of sodium nitrate were added, swirled and then left for few minutes. The extract then cautiously diluted with water. Sometimes the colour develops after warming the mixture. The mixture was made alkaline with 5% NaOH, a colour change was observed in case of phenolics.

3.5.18 Test for Mucilage

Powered drug (seed) material showed red color with ruthenium red making test positive for Mucilage.

3.5.19 Test for Starch

Iodine test: To 3 ml sample solution, few drops of dilute iodine solution were added. Appearance of blue colour, which disappeared on boiling and reappeared on cooling indicated the presence of starch.

3.5.20 Test for Organic acids

Dilute NH_4OH solution was added to neutralized dried plant material extract and then with this the following tests were performed.

Calcium Chloride test: To 2 ml sample solution few drops of 5% $CaCl_2$ solution was added.

(a) Formation of white precipitate indicated the presence of oxalic acid.

(b) Precipitate was observed on shaking or scratching test tube with glass rod denoted the presence of tartaric Acid.

3.5.21 Volatile acids

To a thin section of seed added Sudan III solution, red color obtained by globules indicated presence of volatile oil.

3.6 Quantitative analysis

3.6.1 Determination of Carbohydrates

0.5 g of seed powder was extracted with 80% ethanol. Extract was dissolved in 10 ml water. Different aliquots were prepared and final volume made up to 1 ml by water. Add 5 ml of 96% of concentrated H_2SO_4, followed by shaking and incubation for 40 min. at room temperature. Then 1 ml of 5% phenol was added to each tube and absorbance taken at 490 nm. Standard curve was plotted the using different concentrations from 1 mg/ml glucose stock solution (Krishnaveni *et al.*, 1984).

3.6.2 Determination of Total Flavonoids

The aluminum chloride method was used for the determination of the total flavonoid content of the seed extracts. Aliquots of seed extract were taken and made the volume up to 3ml with methanol. Then 0.1ml $AlCl_3$ (10%), 0.1ml Na-K tartarate and 2.8 ml distilled water were added sequentially and shake vigorously. Absorbance was be recorded at 415 nm after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples was calculated from the calibration plot and expressed as mg quercetin /g of saponin (Khatiwora *et al*., 2010).

3.6.3 Determination of Tannin

2g of seed powder was extracted thrice in 70% acetone. After centrifuging the sample supernatant removed. Different aliquots were taken and made the final volume up to 3 ml with distilled water. The solution after vortexing was mixed with 1 ml of 0.016M $K_3Fe(CN)_6$, followed by 1 ml of 0.02M $FeCl_3$ in 0.10 M HCl. After Vortexing, the

tubes were kept as such for 15 min and then added 5 ml of stabilizer (3:1:1 ratio of water, H_3PO_4 and 1% gum arabic) followed by re-vortexing. Absorbance measured at 700 nm against blank. Standard curve is plotted using various concentrations of 0.001M gallic acid (Graham, 1992).

3.6.4 Determination of glycosides

1g seed powder is weighed and 20 ml 70% alcohol added, and left overnight with occasional shaking for 2 hrs then filtered. Transfer 10 ml of the purified and clear filtrate into a clean dry stoppered erlynmeyer flask and added 10 ml Baljet's reagent. Carried out a blank at the same time, and allowed to stand for one hour (time necessary for maximum color development). Then the solution is diluted with 20 ml distilled water (by automatic burrate) and mixed. Noted the intensity of the color obtained against the blank provide, at 495 nm using a suitable spectrophotometer. The color is stable for several hours. The difference between Experiment and blank (E-B) = original reading.

3.6.5 Determination of Protein

1g seed powder was extracted using 10 ml water, added with few drops of triton X-100. Supernatant was extracted in acetone and the obtained pellet dissolved in 0.1 M NaOH. Aliquots were prepared and final volume made up to 1 ml by distilled water. Add 5 ml of copper reagent to tubes and mixed well, incubated for 10 minutes. 1 ml of *folin's reagent* mixed. After 30 min. incubation at room temperature, absorbance was taken at 700 nm. Plotted the Standard curve using 50 mg/ml BSA (Lowry *et al*., 1951).

3.6.6. Determination of Lipids

1g seed powder was dissolved in ether and stirred for 1 hour. Mixture was centrifuged, supernatant dried and dissolved in ethanol. 0.1 ml of alcohol was taken as blank, olive oil as standard and test sample as unknown respectively. 2 ml of concentrated H_2SO_4 and 5 ml of phosphovanillin reagent were added and mixed well, incubated for 30 min. Absorbance was read at 540 nm (Ganai *et al*., 2005).

3.6.7. Determination of Total Phenolics

0.5-1g seed powder was extracted with 10ml of 80% ethanol. Supernatant was evaporated to dryness and extract was re-dissolved in 5ml water. Different aliquots 0.1-1 ml were pipetted into test tubes and final volume made up to 3ml by water. 0.5 ml of *folin's reagent* followed by 20% Na_2CO_3 equal to 2 ml was added respectively. After vortexing, tubes were kept in boiling water for one minute. After cooling, absorbance was read at 650 nm against blank. A standard curve was prepared using different concentrations of 100 mg/ml catechol (Mallick and Singh, 1980).

3.7 Elemental study

Micro elemental studies of the samples collected from the market and fields were carried out in detail as per the standard methods. Analysis was done at Maharashtra Rajya Draksha Bagaitdar Sangh (MRDBS), a Government of Maharashtra establishment at Manjari Farm, Pune. Maharashtra.

Table 3.1 Elemental study parameters

Sr. No.	Analysis Parameter	Method for Analysis
1	Total Nitrogen	Wet Digestion followed by kjeldahl distillation method
2	Nitrate Nitrogen	Phenol Disulphonic acid method
3. Digestion by Diacid digestion (HNO_3:$HClO_4$-3:1)		
4	Phosphorous	Spectrophotometric vanado Phospho Molybdate Method
5	Potassium	Flame Photometric method
6	Calcium	Atomic Absorption Spectrophotometric method
7	Magnasium	Atomic absorption spectrophotometric method
8	Sulphur	Barium chloride, turbidimetric method
9	Iron	Atomic absorption spectrophotometric method
10	Manganese	Atomic absorption Spectrophotometric method
11	Zinc	Atomic absorption spectrophotometric method
12	Copper	Atomic absorption spectrophotometric method
13	Sodium	Flame photometric method

3.8 Thin layer chromatography (TLC)

TLC is performed on Aluminum packed silica gel $60F_{254}$ TLC plates (Merck). Samples were applied to the plates as sharp bands by means of Camag Linomat IV HPTLC samples applicator. The mobile phase was poured into the chamber left to equilibrate for 30 min. After drying the spots in a current of air the plate was placed in a twin trough glass chamber and developed until the solvent front had travelled a distance of 7 cm above the position of sample application. After that the plate was removed from the chamber and dried in a current of air. The plate was visulalized under UV at 254 nm and 366 nm, and also derivitized by spraying the anisaldehyde sulphuric acid reagent.

$$R_f = \frac{\text{Distance traveled by the substance (cm)}}{\text{Distance traveled by the mobile phase (cm)}}$$

Where "R_f" indicates Retention factor

C. Quantification of L-Dopa in *Mucuna* sps

3.9 Extraction of *Mucuna* sps and L dopa quantitation

Mucuna pruriens, *Mucuna cochinchinensis* black seeds sp. and *Mucuna cochinchinensis* white seeds sp. were used for extraction. The crushed seed powders of these 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, 0.020 mbar) to further concentrate it. The dried, powered forms of these 3 extracts were preserved at dark and cold place to maintain the medicinal value. This powder was used to assess the L-dopa (L-3, 4-dihydroxyphenylalanine) level in it.

A thorough literature survey was done to find out the colorimetric method for quantitating the L-dopa content in the *Mucuna* seeds extracts (Desai *et al*., 2010). 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 shook 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 absorbance of standard L-dopa solutions were recorded at 476 nm (Epoch BioTek, USA). Based on the absorbance obtained for various concentrations of L-dopa standard, calibration plot was made. The standard plot was used for quantitating the L-dopa content in *Mucuna* sps.

D. DNA Fingerprinting by RAPD technique

In the present study, a DNA fingerprinting technique is applied, that is random amplified polymorphic DNA (RAPD), to differentiate the three accessions of *Mucuna*.

3.10 Collection of leaf samples:

The healthy and non-infected leaves of *Mucuna pruriens* (L.) DC., *Mucuna cochinchinensis* (Lour.) A. Chev. Black seed and *Mucuna cochinchinensis* (Lour.) A. Chev. white seed, were collected from the Medicinal plant garden of National Research Institute of Basic Ayurvedic Sciences, Pune. The plant specimens were preserved in the herbarium for future reference.

3.11 DNA isolation:

The leaf samples were grounded in liquid nitrogen into a fine powder and were kept at -20°C until use**.** The total genomic DNA extraction from the leaves of all three plants was done by using 3B Black Biotech Bio tools kit with approximately 100-120 mg of powdered sample of each. The DNA of both plants was then quantified using biophotometer (Eppendorf, Germany) and standardized against known concentrations of DNA marker ladder (Promega, USA) on 1% agarose gels. DNA was then kept at 4°C until further use.

3.12 RAPD- PCR:

RAPD analysis following Williams *et al*., (1990) was employed in order to assess the genetic integrity of *Mucuna* sp. The protocol for polymerase chain reaction (PCR) was optimized by varying the concentration of template, *Taq* DNA polymerase and MgCl2. Polymerase chain reaction (PCR) amplifications were performed in reaction mixtures containing 10 ng template DNA, 0.2 µM primer, 100 µM dNTP´s, 10 mM PCR buffer, 1.5 mM $MgCl_2$, and 1 unit *Taq*-polymerase in a total volume of 20 µl. Amplifications were carried out in a thermo-cycler (Peltier P25+, Cyber lab) with the following program: initial denaturation step of 3 min at 94°C followed by 35 cycles of 45 sec at 94°C, 30 sec at 36°C, 1 min at 72°C, and a final extension step of 5 min at 72°C and 4ºC for the end hold. PCR products were resolved by electrophoresis in 1.5% Agarose gels along with DNA Marker ladder (100-1000bp), the run was

carried out at 75 Volts for approximately 60 minutes. Gels were stained with ethidium bromide, visualized under UV-light, and photographed GelDoc system (BioRad, USA). To ensure reproducibility of banding patterns, each amplification reaction was repeated twice. All the PCR reaction components and the Primers set were purchased from 3B Blackbio Biotech Biotools. The primers used for amplification of the genomic DNA were detailed in Table 3.1 Along with the 25 different reaction mixtures for 25 different primers, a negative control (NC - No DNA template) and a positive control (PC - No primer) were placed in thermal cycler to rule out the possibility of contamination. Reaction mixture wherein template DNA replaced by distilled water was used as negative control.

Table 3.2: Plant RAPD primers used for the study

No.	Primer code	Accession Numbers	No.	Primer code	Accession Numbers
1	RPl 1	AM765819	14	RPl 14	AM773774
2	RPl 2	AM750044	15	RPl 15	AM773775
3	RPl 3	AM773310	16	RPl 16	AM773776
4	RPl 4	AM773769	17	RPl 17	AM911710
5	RPl 5	AM773770	18	RPl 18	AM765830
6	RPl 6	AM773771	19	RPl 19	AM773777
7	RPl 7	AM773312	20	RPl 20	AM773317
8	RPl 8	AM773773	21	RPl 21	AM765820
9	RPl 9	AM773315	22	RPl 22	AM911711
10	RPl 10	AM750045	23	RPl 23	AM911712
11	RPl 11	AM911709	24	RPl 24	AM765821
12	RPl 12	AM773316	25	RPl 25	AM750054
13	RPl 13	AM750046			

3.13 Statistical Analysis:

Gelquest® and Clustervis® software were used to construct dendro-grams using the Unweighted Pair Group Method with Arithmetical Averages (UPGMA) by comparing the bands that were observed common in both plants to specific primers.

E. Pharmacology and Toxicology study

3.14 Antioxidant activity

I. Determination of flavonoids:

When flavonoids react with aluminium chloride it forms an acid stable complex with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. Total Flavonoids content in seed extracts was determined according to the method described by Khatiwora *et al.* (2010). The absorbance is measured at 415 nm against the standard quercetin. Various concentrations of standard quercetin solution were prepared to make a standard calibration curve. The concentration of flavonoid in the test sample was calculated from the calibration plot.

II. 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 (Slinkard, 1977). 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 seed extracts and standard Gallic acid dissolved in distilled water. After 5 min incubation at room temperature, 80 µl of sodium carbonate (75 g/L) was 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, USA). The assay was repeated thrice and Total phenolic contents (average of three) were expressed as Butylated Hydroxy Toluene equivalent per gram of lyophilized extract.

III. Inhibition of DPPH radical:

The free radical scavenging activity of extracts were measured by 1; 1-diphenyl-2-picryl-hydrazil (DPPH) as a method described by Blois (1958). 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).

IV. Reducing power/Ferric reducing antioxidant potential (FRAP) assay:

The reducing power of seed extracts was determined by using the method of Oyaizu (1986). 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 [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50ºC 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 ($FeCl_3$) (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 graphs were plotted with the average of three observations.

3.15 Animal Experimentation

In this study Sprague Dawley strain rats were used. Male and female rats weighting around 160 to 270 g were procured. This work was carried out at Toxindia Contractual Research Organization, Pune, after getting approval of Institutional Animal Ethics Committee (IAEC) meeting held on 26th September 2013, with approval code number TI003 as per guidelines of the Committee for the Purpose of Control and Supervision of Experimentations on Animals (CPCSEA). The rats were acclimatized for one week in experimental room after veterinary examination. The animals were housed in clean poly propylene cages with stainless steel grill top, with facilities for food and water bottle, and bedding of clean paddy husk. The environmental conditions such as air conditioned experimental rooms with 10-15 air changes per hour, temperature between 21±2^{0}C, relative humidity 55±5% and illumination cycle set to 12 hours fluorescent light and 12 hours dark. Diet of the animals is “Nutrimix” brand pelleted standard rat and mice feed Manufactured by Nutrivet Life Sciences, Pune, was provided *ad libitum*. Potable water passed through reverse osmosis filtration system and exposed to U.V. ray was provided *ad libitum* in glass bottles with stainless steel sipper tubes.

3.15.1 Spermatogenesis evaluation:

This study was performed to investigate the effects of *Mucuna cochinchinensis* (Black seed), *Mucuna cochichinensis* (White seed) and *Mucuna pruriens* on semen quality in albino Sprague Dawley rats. Sixty six male rats weighing between 250-270 g were used. They were divided into 11 groups of 6 rats in each group.

Group I (Control) received distilled water and normal rat chow. Group II (Standard) received L-Dopa at 20 mg/kg. While test groups III, IV, and V received 100, 200 and 400 mg/kg of *Mucuna cochinchinensis* (Black seed) seed extract. Similarly Group VI, VII, and VIII, received 100, 200, and 400 mg/kg of *Mucuna cochichinensis* (White seed) seed extract, and Group IX, X, XI received 100, 200, and 400 mg/kg of *Mucuna pruriens* seed extract for 30 days. Blood samples were collected from all animals per group and preserved for analysis. Semen from epidydimis was collected and analyzed for sperm count, sperm motility and morphology.

Study design:

Groups of each with 6 male Sprague Dawley rats have received standard drug and different doses of sample compounds for 30 days. Animals were sacrificed on 31st day for analysis and evaluation of its effects. Control group received only water for 30 days.

Experimental procedure:

Sample	Group number	Dose level mg/kg/day	Animal Numbers per group
Normal Control	I	----	6
L-Dopa	II	20	6
Seed extract of *Mucuna cochichinensis* (Black seed)	III	100	6
	IV	200	6
	V	400	6
Seed extract of *Mucuna cochichinensis* (White seed)	VI	100	6
	VII	200	6
	VIII	400	6
Seed extract of *Mucuna pruriens*	IX	100	6
	X	200	6
	XI	400	6

Method of administration:

The method of administration was oral by gavage. Based on human exposure the dosage route was selected. The sample materials were given for 30 days, daily. Dosage volumes were calculated and adjusted daily for individual animals.

Observations:

Mortality:

During the entire study the animals were checked daily on morning, afternoon and evening as well for any dead or morbidity among them.

General clinical examination:

The rats were subjected to general cage side examination on each day at suitable intervals after dosing with sample material.

Detailed Clinical Examination:

The rats were subjected to detailed clinical examinations before initiation of the treatment and daily thereafter during the treatment period. These observations were made outside the home cage in a standard area and preferably at the same time. Signs noted were including changes in the skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity such as lacrimation, piloerection, pupil size, unusual respiratory pattern, etc. Changes in gait, posture and response to handling as well as presence of clonic or tonic movements, stereotypes or bizarre behavior if any also recorded.

Body weight:

The body weight of each rat was recorded at the time of selection of the animal to include into the groups *i.e* one day prior to the day of commencement of treatment, weekly thereafter and lastly at the time of necropsy.

Food consumption:

The quantity of food consumed by each rat in cage was recorded on the day of commencement of treatment and weekly thereafter. Food intake was calculated using the food offered to and left in the cage.

Terminal studies:

Necropsy examination:

On completion of 30 days of treatment, all surviving rats were sacrificed by exsanguinations method under carbon dioxide anesthesia and were subjected to complete necropsy. The testis and epididymis were excised, freed from the attached fat and connective tissue.

Sperm count:

The Epididymis was minced with anatomic scissors in 5 ml physiologic saline and placed in a rocker for 10 minutes and allowed to incubate at room temperature for 2 minutes. After incubation, the supernatant fluid was collected and diluted 1:100 with solution containing 5 g of sodium bicarbonate and 1 ml formalin (35%). Total sperm count was determined by using the new improved Neuber's counting chamber (Haemocytometer). Approximately 10µL of the diluted sperm suspension was transferred to each counting chamber of the haemocytometer and allowed to stand for 5 minutes. This chamber was then placed under a binocular microscope having an adjustable light source. The ruled part of the chamber was then focused and the number of spermatozoa counted in five 16-celled squares. The sperm concentration was the calculated number multiplied by 5 and expressed as [X] x 106 /ml, where [X] is the number of spermatozoa in a 16-celled square.

Sperm progressive motility:

The fluid obtained from the left *cauda* epididymis with a pipette was diluted to 0.5 ml with Tris buffer solution (pH 7.5). An aliquot of this solution was placed on the slide and slide was placed on light microscope with temperature controlled at 37ºC. The microscopic field was scanned systematically and each spermatozoon encountered was assessed, for the purpose of the first part of the assessment. Motility was classified as either motile or non-motile and percentage motility was evaluated visually at a magnification of 400X. Motility estimates were performed from three different fields in each sample. The mean of the three estimations was used as the final motility.

Sperm morphology:

The sperm cells were evaluated with the aid of light microscope at 400X magnification. Caudal sperm was taken from the original dilution for motility and further diluted to 1:20 with 10% neutral buffered formalin (Sigma- Aldrich, Oakville, ON, Canada). Five hundred sperms from the sample were scored for morphological abnormalities. Briefly, in wet preparations using phase-contrast optics, spermatozoa were categorized. In this study, a spermatozoon was considered morphologically abnormal if it has one or more of the following features, like rudimentary tail, round head and detached head. Abnormality was expressed as a percentage of morphologically normal sperm.

Blood samples:

Blood samples were collected by orbital bleeding from anesthetized animals after 30 days. Approximately 1-1.5 ml of blood was collected from each rat in each group including the control group. The samples were carefully introduced into containers free from anticoagulant and properly labeled for example 1a, 1b, 1c and 1d. The blood samples are allowed to clot, retract and then centrifuged for 5minutes at a speed of 5000 revolutions per minute. The plasma was collected, refrigerated at -20°C for further analysis.

Statistical analysis:

The results were expressed as mean ± SEM. The significance of differences were analyzed by using Student's t-test, $P < 0.05$ was considered as significant.

3.15.2 Toxicological evaluation

I. Acute Toxicity:

Objective of this Acute oral toxicity study is to assess toxic effects of 3 sample materials that is seed powder extracts of *Mucuna cochinchinensis* (Black seed), *Mucuna cochichinensis* (white seed) and *Mucuna pruriens*, in rats. In this study assessment was to see and record the toxic characteristics shown by them when sample material was administered as a single oral dose as per the OECD 423 guidelines.

Test system : Rat (Sprague Dawley strain)

Source : Bred and reared at Toxindia, Pune.

Age : 8 to 9 weeks.
Body weight range at initiation: 168 to 178 g
Identification : By cage tag and corresponding color body markings.
Number of dose groups for
Each compound material : Three
G1: Vehicle Control,
G2: 2000 mg/kg,
G3: 2000 mg/kg.
Number and sex of animals
in the study : Three females
Nutritional conditions : Fasted for four hours prior to treatment. Food was offered one hour after dosing.

Study design:

Principle of the test:

It is the principle of the test that is based on a stepwise procedure with the use of a minimum number of animals per step. Sufficient information is obtained on the acute toxicity of the sample material to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of sample material related mortality of the animals dosed at one step will determine the next step, that is

- no further testing is needed,
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level.

Single animals were dosed in sequence usually at 48 hrs intervals. However, the time intervals between dosing were determined by the onset, duration, and severity of toxic signs. Dosing of an animal at the next dose was delayed until one was confident of survival of the previously dosed animal. The time interval adjusted as appropriate, example in case of inconclusive response. The test is simpler to implement when a single time interval is used for making sequential dosing decisions.

Dose levels and justification:

A starting dose level of 2000 mg/kg body weight was selected from one of the four fixed levels (5, 50, 300 and 2000 mg/kg) based on the available historical toxicity data of the *Mucuna cochinchinensis* (Black seed), *Mucuna cochichinensis* (White seed) and *Mucuna pruriens.* Food was withheld for overnight prior to administration of the sample material. However, rats had an access to water *ad libitum.*

The toxicity of the samples following oral gavages were assessed by stepwise treatment of animals. Three female rats were used per step. The rats were observed for incidence of mortality and signs of intoxication for 14 days after the administration of test article. Further, the test was repeated on three more female rats at the same dose level and observed for 14 days.

Dose preparation

The seed extract of *Mucuna cochinchinensis* (Black seed), *Mucuna cochichinensis* (White seed) and *Mucuna pruriens* were suspended separately in distilled water to obtain desired concentration as mg/ml just before dosing every day.

Administration of sample material

The sample materials of *Mucuna cochinchinensis* (Black seed), *Mucuna cochichinensis* (White seed) and *Mucuna pruriens* at the prescribed concentration was administered to each rat by single oral gavage using 18G stainless steel intubation needle fitted onto a suitably graduated syringe. Food was with held overnight prior to the administration of sample material, however water was made available *ad libitum* during this period. The rats were then observed for incidence of mortality and signs of intoxication for 14 days.

Observations

Mortality

On the day of dosing, all animals were observed for mortality at 30 min, 1, 2, 3, 4 and 6 hours after dosing and thereafter they were observed once in a day for 14 days.

Clinical signs

The treated animals were observed for signs of intoxication, at 30 min, 1, 2, 3, 4 and 6 hours after dosing and thereafter once a day for 14 days. The appearance, progress and disappearance of these signs were recorded.

Body weights

The body weights of rats were individually recorded before dosing and at weekly intervals thereafter.

Necropsy and Histopathology

All animals were subjected to gross necropsy. As no gross pathological findings were encountered in any of the organs, histo-pathological examination was not conducted.

II. Sub-acute Toxicity:

The objective of this 'Repeated Dose 28-Day Oral Toxicity Study with 14 days exposure period in Sprague Dawley rat is to assess the toxicological profile of aqueous seed extract of *Mucuna cochinchinensis* (Black seed) when administered daily, over a period of 28 days. This study is aimed to get the information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time.

Test species		: Rat
Strain of the species		: Sprague Dawley.
Age at start of study		: 7 to 8 weeks.
Weight range		:
Male rats	Mean	: 178.06(100%)
		Minimum : 170 (-4.83%)
		Maximum : 186 (+4.20%)
		n : 30
Female rats	Mean	: 169.4 (100%)
		Minimum : 160 (-5.57%)
		Maximum : 175 (+ 3.29%)
		n : 30
Number of animals per dose group		: Ten (Five male and Five female)

Study design:

Groups of five male and five female Sprague Dawley rats were administered aqueous seed extract of *Mucuna cochinchinensis* (Black seed) by oral gavage daily at the doses of 0mg/kg, 0mg/kg-R, 250 mg/kg, 500 mg/kg, 1000 mg/kg and 1000 mg/kg-R body weight for 28 days and were sacrificed on day 29 to evaluate its toxicity. Concurrent control group receiving the vehicle was also maintained.

Dose Group	Treatment level (mg/kg)	Treatment (28 days)	
		Male	Female
G1	0 (Vehicle control)	5	5
G2	0 Control reversal	5	5
G3	250 Low Dose	5	5
G4	500 Intermediate Dose	5	5
G5	1000 High Dose	5	5
G6	1000 High reversal	5	5
Total: 60 Rats (30 Males + 30 Females)			

Administration of test material:

The aqueous extract of *Mucuna cochinchinensis* (Black seed) was administered to animals by orally at the same time each day wherever was possible, using a suitably graduated syringe and intubation needle (18G). The dosage volume administered to individual rat was adjusted according to its most recently recorded body weight. The control group rats received the vehicle (Distilled water) only, by oral gavage at the same dosage volume of 10ml/kg body weight. Treatment in this manner was continued once a day, seven days a week, for a total period of 28 days.

Observations:

Mortality:

Throughout the study, all animals were checked early on each working day and again in the afternoon to look for dead or morbid animals to allow necropsy examination to be carried out during the working hours of that day. At weekends and

public holidays a similar procedure is followed except that the final check was carried out at approximately mid-day.

Clinical Signs:

All signs of ill health, together with any behavioural changes or reaction to treatment were recorded for every individual animal.

General Clinical Examinations :

The rats were daily subjected to general cage side clinical examinations, at the same time each day and at suitable intervals after dosing, considering the peak period of anticipated effects after dosing.

Detailed clinical examination:

The rats were subjected to clinical examinations before initiation of the treatment and daily thereafter during the treatment period. These observations were made outside the home cage in a standard arena and preferably at the same time. Observations noted were including changes in the skin, fur, eyes, mucous membranes, occurrence of various secretions and excretions. Autonomic activity like lacrimation, piloerection, pupil size, respiratory pattern, etc was also examined. Changes in gait, posture and response to handling as well as presence of clonic or tonic movements, stereotypes or bizarre behavior, if any, also recorded.

Functional Observations :

In the fourth week of exposure, all rats were examined for assessment of sensory reactivity, assessment of grip strength and motor activity. These include the functional observation battery suggested by Gad (1982) and Moser *et al.* (1991).

Body weight :

The body weights of rats were individually recorded before dosing and at weekly intervals after dosing.

Food Consumption:

The quantity of food consumed by rats in each cage was recorded on the day of commencement of treatment and weekly after that. Food intake per rat was

calculated using the amount of food offered to and left in each cage after 24 hours, and the number of rats surviving in each cage.

Pathology:

Clinical Pathology:

Samples of blood were collected on termination of treatment, on 29th day under carbon dioxide anaesthesia, from the retro-orbital plexus of all the male and female rats. The samples were collected in tubes containing Heparin (for clinical chemistry) and K-EDTA (for haematology) as an anticoagulant.

Food was removed overnight from animals to be sampled for laboratory investigations. Blood smears were made on glass slides. Clinical chemistry samples were centrifuged at a speed of 5000 rpm for 5 minutes.

The tests that were performed on blood samples have been listed below, together with an abbreviated title (used in Appendices and Tables).

Haematology:

The following estimations, with their units of measurement as listed below, were performed using 'Coulter A_C.T diff' Haematology Analyser (Beckman Coulter, Inc., Miami, Florida, USA):

Haemoglobin (Hb) (g/dl)

Packed cell volume (PCV) (%)

Total red cell count (total RBC) (x10^6/cmm)

Total white cell count (Total WBC) (x10^3/cmm)

Platelet Count, Total (Platelets) (x10^3/cmm)

Following absolute erythrocyte indices are calculated:

Mean Corpuscular volume (MCV) (fl)

Mean corpuscular Haemoglobin (MCH) (pg)

Mean corpuscular haemoglobin concentration (MCHC) (g/dl)

Clotting time measurement (seconds) was performed manually using standard techniques.

Differential WBC counts were determined by microscopy of blood smear, stained with Wright's stain, counting 100 cells -

Neutrophils (N) %, Lymphocytes (L)%, Eosinophils (E) % and Monocytes (M)%

Clinical Chemistry:

Plasma chemistry parameters, with their units of measurement as listed below, were analysed using the "Erba Smartlab Random Access Batch Analyser / Erba EC5 Plus Analyser" (Transasia Bio-Medicals Ltd., India) using standard methodology:

Total protein (g/dl), Albumin (g/dl), Globulin (calculated as g/dl), Alanine aminotransferase (ALT, IU/L), Aspartate aminotransferase (AST, IU/L), Glucose (mg/dl), Alkaline phosphatise (ALP, IU/L), Total Cholesterol (CHOL, mg/dl), Triglycerides (mg/dl), Creatinine (CR, mg/dl), Bilirubin (mg/dl), Phosphorous (P, mg/dl), Calcium (Ca, mg/dl).

Following electrolytes were assayed using EasyLyte Na/K Analyser (Medica Corporation, Bedford, MA, USA) employing ion selective electrodes:

Sodium (mmol/L), Potassium (mmol/L)

Urinalysis:

Urinalysis was performed on all animals in last week of the study before termination of the treatment period (week 4).

Urine samples are collected using a battery of specially designed stainless steel urine collection cages. Each rat was housed in this cage. Urine samples were collected over a period of 4 hours. Food and water was not offered during this period.

Tests were performed using Multistix® SG Multiple Reagent Diagnostic Strips manufactured by Bayer Diagnostics India Ltd., Baroda, India and were used as qualitative / semi-quantitative indicators of analyte concentration.

Qualitative tests:

Colour, Appearance, Specific gravity, pH, Protein and/or Albumin, Glucose, Ketone, Bilirubin, Urobilinogen, Occult Blood.

Microscopy:

For microscopic examination, a drop of urine sample was spread on a microscope slide and examined for the presence of the following constituents :

Epithelial cells (E), Leucocytes (P), Erythrocytes (R), Casts (C), Crystals.

Terminal Studies:

Necropsy:

On completion of 28 days of treatment, all surviving rats were sacrificed by exsanguinations manner under carbon dioxide anaesthesia and were subjected to complete necropsy. The necropsy performed at termination of the treatment was conducted on day 29. All the tissues *viz.* Heart, liver, kidney, spleen, testes (male), ovaries (female), stomach, intestine, adrenals, brain, thymus, uterus (female), epididymis (male), from all animals, were preserved, in 10% neutral buffered formalin. In addition, samples of any macroscopically abnormal tissues were routinely preserved, along with samples of adjacent tissue.

Organ Weights:

Below mentioned organs, from all animals sacrificed, were dissected free from fat and weighed wet as soon as possible to avoid drying: Kidneys, liver, adrenals, testes / ovaries, spleen, brain and heart.

Histopathological Examination:

Tissues that were subjected to microscopic examination in this study were Heart, liver, kidney, spleen, testes (male), ovaries (female), stomach, intestine, adrenals, brain, thymus, uterus (female), epididymis (male). These tissues were embedded in paraffin wax, sectioned at five micrometers and stained with haematoxylin and eosin. A full histopathological examination was conducted on the specified list of tissues.

Microscopic examination was not extended to tissues of lower dose levels in absence of any treatment related alterations being detected at the high dose level.

Statistical analysis:

ANOVA was performed on homogenous set of data to determine the treatment effects . Dunnett's and Bartlett’s test was also used for statistical analysis of the data as appropriate. The level of significance for variance was analyzed at 5%.

Chapter 4: Observations and Results

A. Pharmacognostical study

4.1 Pharmacognostic evaluation of *Mucuna pruriens* (L.) DC.

4.1.1 Macroscopy

Seeds of *Mucuna pruriens* (L.) DC. are ovate, oblong, flattened, 0.8 to 1.29 cm in length, 0.6 to 0.9 cm in breadth and 0.3 to 0.63 in thickness. Surface hard, smooth, glossy, dark brown spotted black. Hilum conspicuous/ funicular 0.4 to 0.5 cm long, more or less elongated with indistinct slit, cushioned with white spongy tissue and bordered with aril located at one of the longer edge of seed near micropylar end.

4.1.2 Microscopy

Diagrammatically T. S. of seed is oval and L.S. is oblong to oval in shaped.

Testa: Detailed T.S. passing through centre of seed shows the outermost malpighian layer of testa composed of thick walled, cylindrical palisade cells, with narrow lumen, often filled with brown colouring matter, through which a light line linea lucida extend across, covered with thin cuticle, underneath this lies a layer of sub epidermis consisting of spool or bone shaped cells with broad apical and basal ends connecting with long vertically running narrow strips, followed by 9 to 15 rows of spongy parenchyma with somewhat thickened walls, the cells of the outer region are big in size with large intercellular spaces unlike the cells of the middle region which are smaller in size, with few intercellular spaces, embedded with vascular strands and the cells of the innermost tissue being obliterated.

Cotyledon: Consists of upper and lower epidermis embedded with aleurone grains; enclosing the wide zone of mesophyll containing starch grains, aleurone grains. Mesophyll cells traverse with developed and undeveloped vascular bundles.

Hilum: Section passing through the hilum differ from the above in following respect: It shows two rows of palisade cells underneath the spongy parenchyma of hilum cushion, lying adjacent to the arillus, sub epidermis forms a thick, loose tissue of variously shaped cells with dark contents, embedded with tracheidal bar and recurrent vascular bundles, a narrow groove run from the centre of the two rows of palisade cells, connecting the tissue of the tracheidal bar.

Plate -1: Morphology of *Mucuna pruriens* (L.) DC.



Habit



Flowering twig



Fruiting twig



Dry pod



Dry Seeds



Seed Powder

4.1.3 Powder Microscopy (Organoleptic character)

Seed powder is creamish gray in colour, coarse, odourless, and taste starchy and slightly bitter at end. Seed powder shows the characters like

1. Fragment of Palisade cells in surface view seen from above.
2. Fragment of Palisade cells in surface view seen from below.
3. Fragment of Palisade in sectional view from testa.
4. Fragment of double layered Palisade in sectional view from Hilum region.
5. Isolated fragments of spoon shaped cells in sectional view.
6. Fragment of spool shaped cells in surface view.
7. Fragment of tracheidal bar.
8. Spiral vessels
9. Isolated starch grain from cotyledon.
10. Isolated starch grains from radical.
11. Fragment of cotyledon showing aleurone grains and starch grains.
12. Fragment of radical showing aleurone grains and starch grains.
13. Isolated cells of Arillus.
14. Fragment of irregular parenchyma from hilum region.

Plate 2: Section passing through Testa of *Mucuna pruriens* (L.) DC.

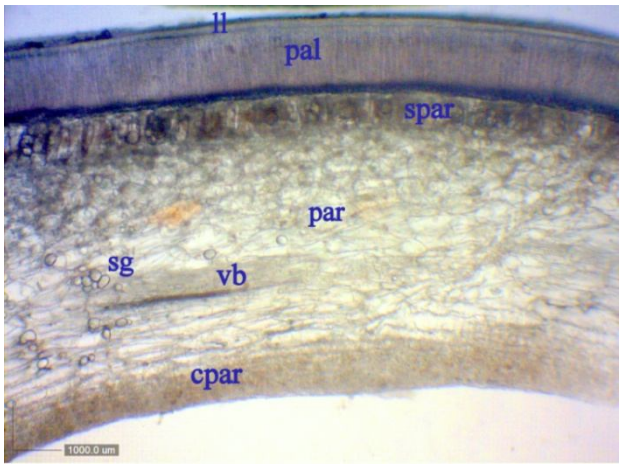


Fig. a

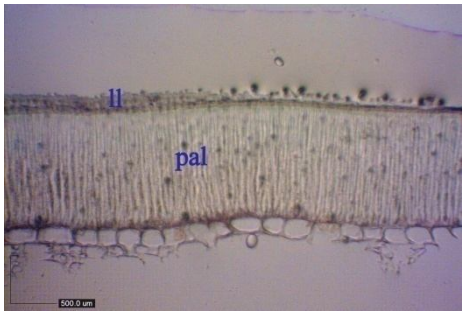


Fig. b

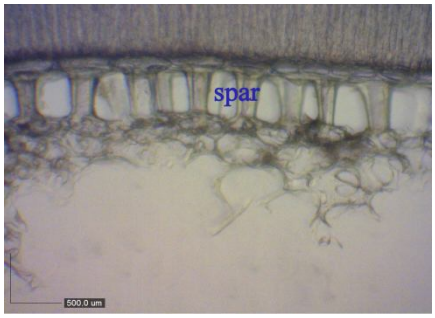


Fig. c

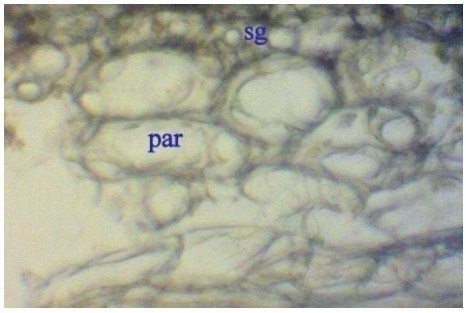


Fig. d

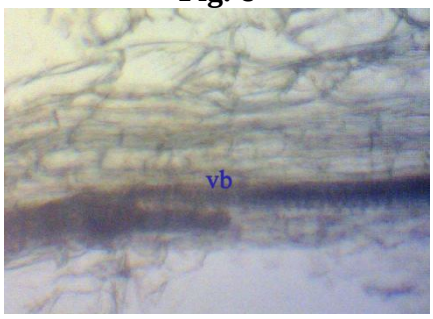


Fig. e

Fig a.: Detailed transverse section of seed passing through testa.
Fig b.: Enlarge view of palisade showing linea lucida.
Fig c.: Enlarge view of spool shaped parenchyma.
Fig d.: Enlarge view of testa showing parenchyma.
Fig e.: Enlarge view of testa showing parenchyma transverse with vascular bundle.

Abbreviations-
cpar - collapsed parenchyma; ll - linea lucida; pal – palisade; par – parenchyma; sg - starch grains; spar - spool shaped parenchyma; vb - vascular bundle

Plate 3: Section passing through Cotyledon and Radical of *M. pruriens*

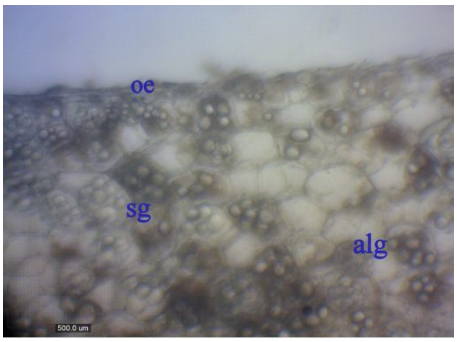


Fig. a

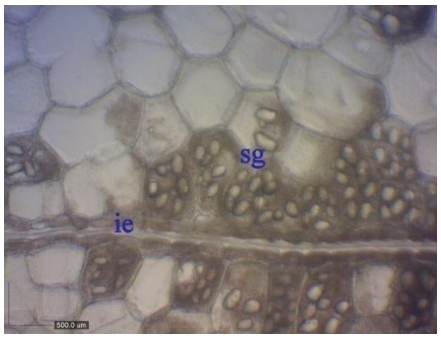


Fig. b

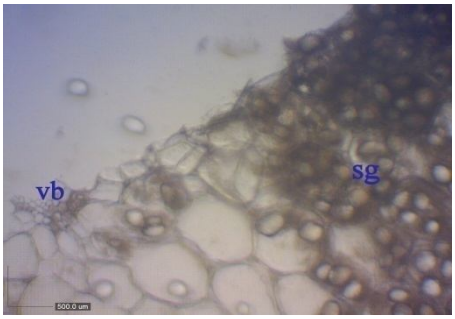


Fig. c

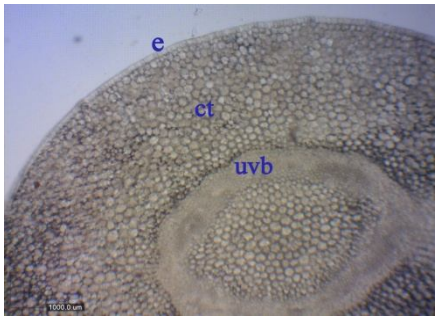


Fig. d

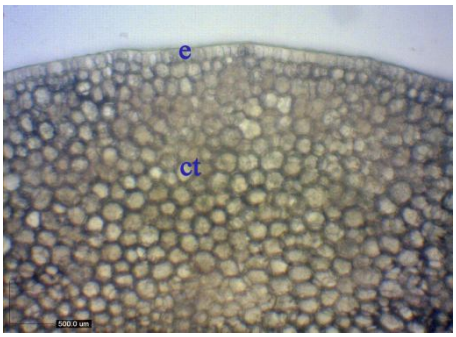


Fig. e

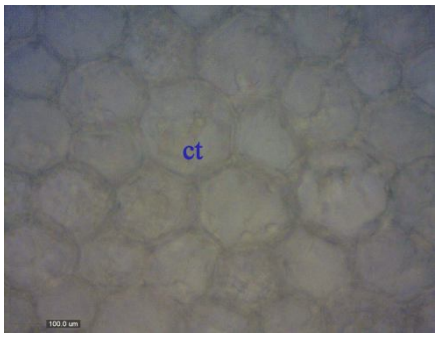


Fig. f

Fig. a: Section passing through cotyledon showing outer epidermis filled with starch grains and aleurone grains.

Fig. b: Section passing through cotyledon showing inner epidermis filled with starch grains and aleurone grains.

Fig. c: Section passing through cotyledon showing middle mesophyll cells filled with starch grains, aleurone grains and traverse with undeveloped vascular bundle.

Fig. d: Section passing through the radical.

Fig. e: Section passing through the radical showing epidermis and cortex region.

Fig. f: Enlarge view of cortex

Abbreviations-

alg – aleurone grain; ct – cortex; e – epidermis; ie – inner epidermis; oe – outer epidermis; sg – starch grains; uvb – undeveloped vascular bundle; vb – vascular bundle

Plate 4: Section passing through Hilum of *M. pruriens*

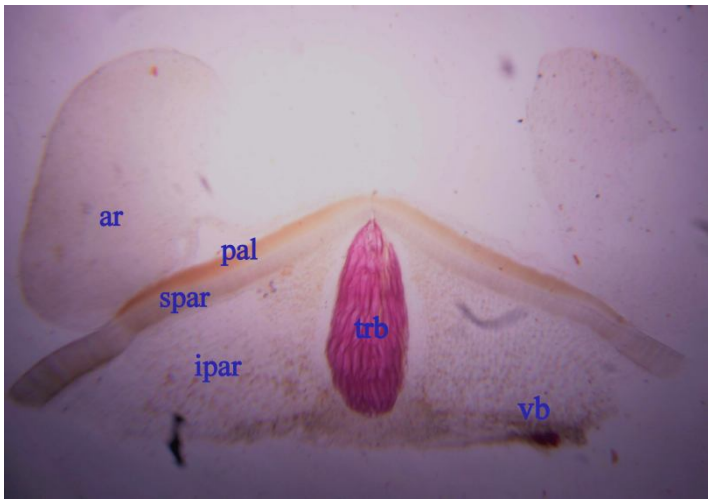


Fig. a

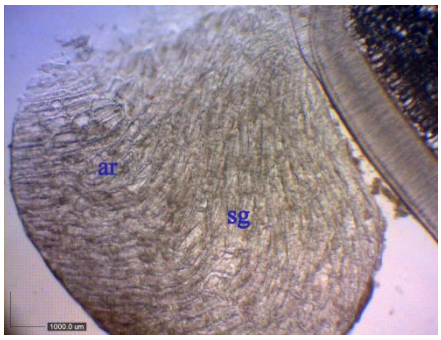


Fig. b

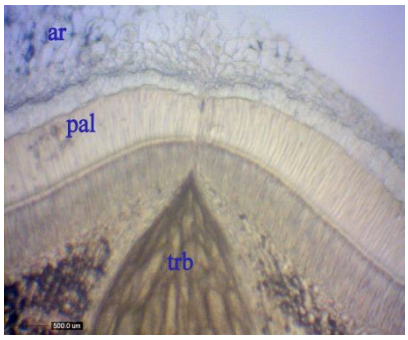


Fig. c

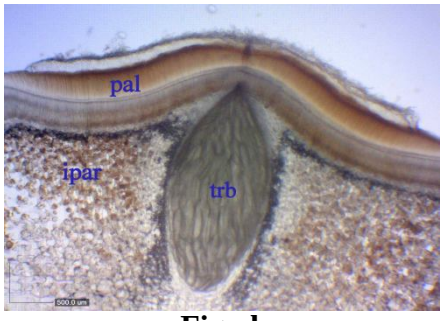


Fig. d

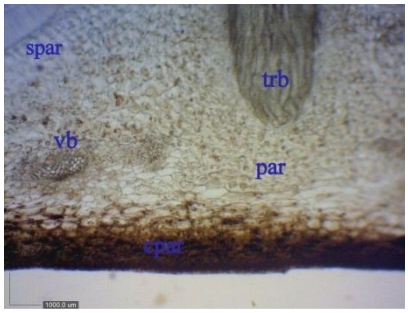


Fig. e

Fig. a: Detailed transverse section of seed passing through Hilum
Fig. b: Enlarge view of section showing Arillus.
Fig. c: Enlarge view of section showing double layered palisade cells.
Fig. d: Enlarge view of section showing tracheidial bar and irregular parenchyma.
Fig. e: Enlarge view of section showing lower parenchyma and vascular bundle.

Abbreviations-
ar- arillus; cpar- collapsed parenchyma; ipar- irregular parenchyma; pal- palisade; par- parenchyma; sg- starch grains; spar- spool shaped parenchyma; trb- tracheidal bar; vb- vascular bundle

Plate 5: Powder Characters of *M. pruriens*

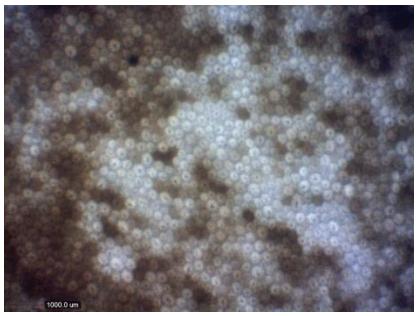


Fig. a

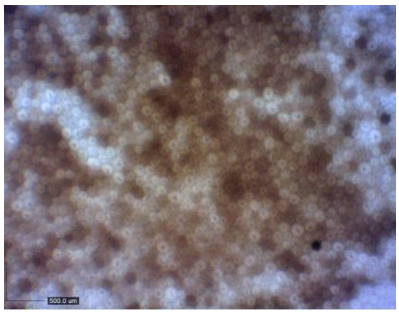


Fig. b

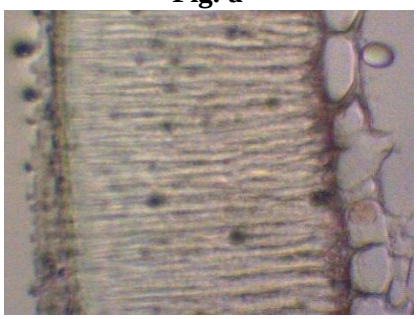


Fig. c

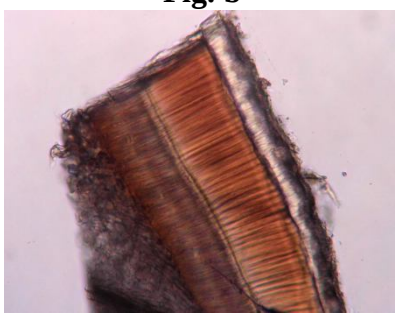


Fig. d

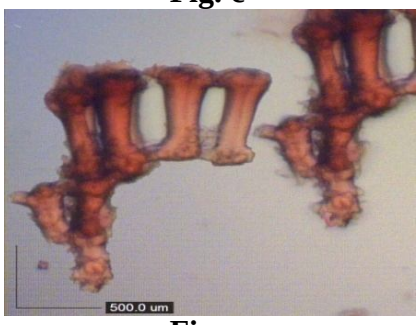


Fig. e

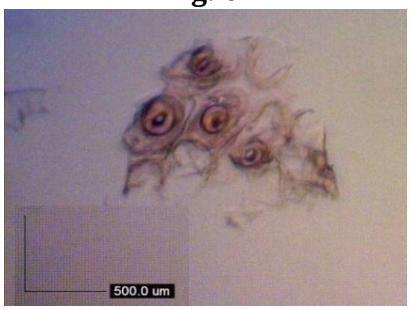


Fig. f

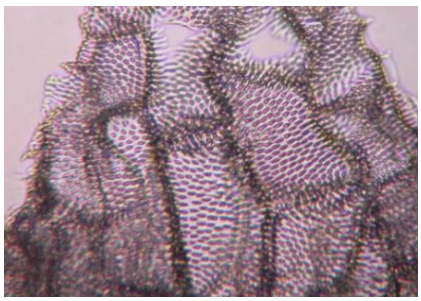


Fig. g



Fig. h

Fig. a: Fragment of Palisade cells in surface view seen from above.
Fig. b: Fragment of Palisade cells in surface view seen from below.
Fig. c: Fragment of Palisade in sectional view from testa.
Fig. d: Fragment of double layered Palisade in sectional view from Hilum.
Fig.e: Isolated fragments of spool shaped cells in sectional view.
Fig. f: Fragment of spool shaped cells in surface view.
Fig. g: Fragment of tracheidal bar.
Fig. h: Spiral vessels

Plate 6: Powder Characters of *M. pruriens*



Fig. a

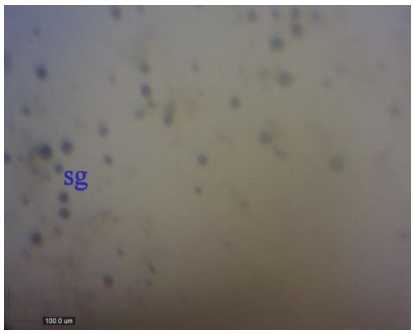


Fig. b

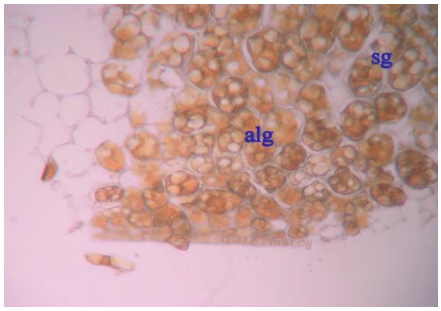


Fig. c

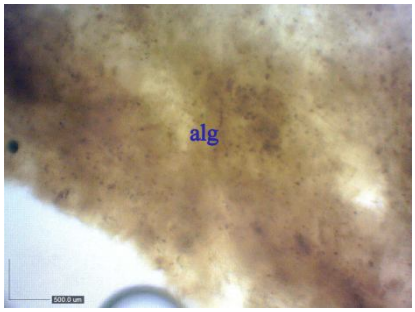


Fig. d

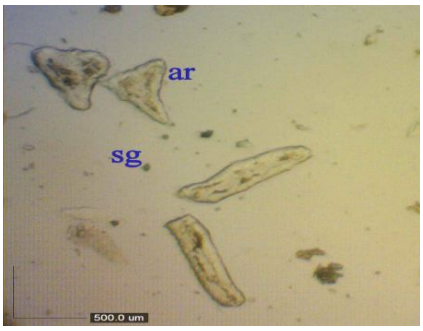


Fig. e

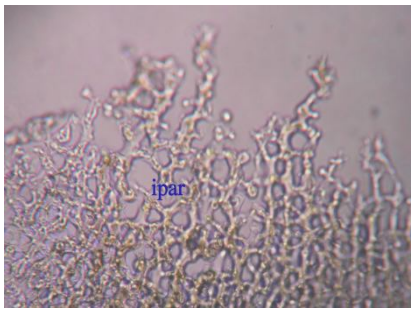


Fig. f

Fig. a: Isolated starch grain from cotyledon.
Fig. b: Isolated starch grains from radical.
Fig. c: Fragment of cotyledon showing aleurone grains and starch grains.
Fig. d: Fragment of radical showing aleurone grains and starch grains.
Fig. e: Isolated cells of Arillus.
Fig. f: Fragment of irregular parenchyma from hilum region.

Abbreviations-
alg- aleurone grains; ar- arillus; ipar- irregular parenchyma; sg- starch grains

4.2 Pharmacognostic evaluation of *Mucuna cochinchinensis* (Lour.) A. Chev. (White Seed)

4.2.1 Macroscopy

Seeds are ovate, oblong, globose, 1.20 to 2 cm in length, 0.95 to 1.37 cm in breadth and 0.50 to 1 cm in thickness. Surface hard, smooth, glossy, dull white with black spots. Hilum conspicuous, 0.7 to 0.9 cm long, more or less elongated with indistinct slit, cushioned with white spongy tissue and bordered with aril located at one of the longer edge of seed near micropylar end.

4.2.2 Microscopy

Testa: Diagrammatically T. S. of seed is oval and L.S. is oblong to oval in shaped. Detailed T.S. passing through centre of seed shows the outermost malpighian layer of testa composed of thick walled cylindrical palisade cells with narrow lumen broadest at base, often filled with brown colouring matter, through which a light line linea lucida extend across, covered with thin cuticle, underneath this lies a layer of sub epidermis consisting of spool or bone shape cells with broad apical and basal ends connecting with long vertically running narrow strips followed by 9 to 15 rows of spongy parenchyma with somewhat thickened walls. The cells of outer region are big in size with few intercellular spaces, embedded with vascular strands and the cells of the innermost tissue being obliterated.

Cotyledon: Cotyledon consists of upper and lower epidermis embedded with aleurone grains. Mesophyll cells traverse with developed and undeveloped vascular bundles.

Hilum: Section passing through the hilum differ from the above in following respect: It shows two rows of palisade cells underneath the spongy parenchyma of hilum cushion, lying adjacent to the arillus, subepidermis forms a thick, loose tissue of variously shaped cells with dark contents, embedded with tracheidal bar and recurrent vascular bundles, a narrow groove run from the centre of the two rows of palisade cells connecting the tissue of the tracheidal bar.

Plate 7: Morphology of *Mucuna cochinchinensis* (Lour.) A. Chev. (White Seed)



Habit



Flowering twig



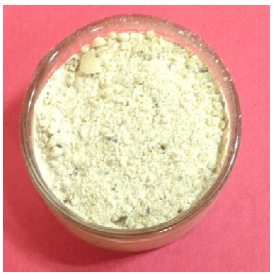
Fruiting twig



Dry pod



Dry Seeds



Seed Powder

4.2.3 Powder Microscopy: (Organoleptic character)

Seed powder is yellowish cream in colour, coarse, odourless, and taste starchy and slightly bitter. Seed powder shows the characters like

1. Fragment of Palisade cells in surface view seen from above.
2. Fragment of Palisade cells in surface view seen from below.
3. Fragment of Palisade in sectional view from testa.
4. Fragment of double layered Palisade in sectional view from Hilum region.
5. Isolated fragments of spool shaped cells in sectional view.
6. Fragment of spool shaped cells in surface view.
7. Fragment of tracheidal bar.
8. Spiral vessels
9. Isolated starch grain from cotyledon.
10. Isolated starch grains from radical.
11. Fragment of cotyledon showing aleurone grains and starch grains.
12. Fragment of radical showing aleurone grains and starch grains.
13. Isolated cells of Arillus.

Plate 8: Section passing through Testa of *M. cochinchinensis* (White Seed)

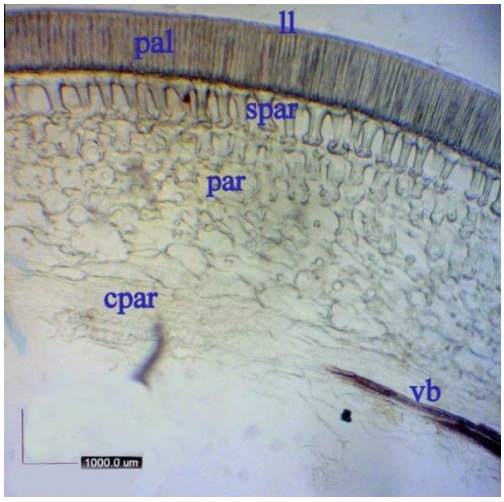


Fig. a

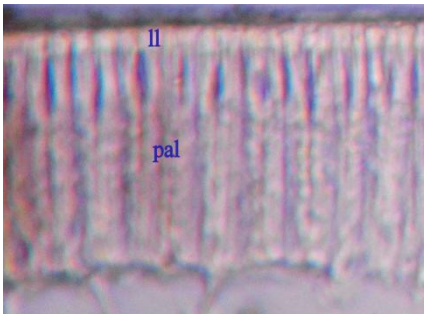


Fig. b

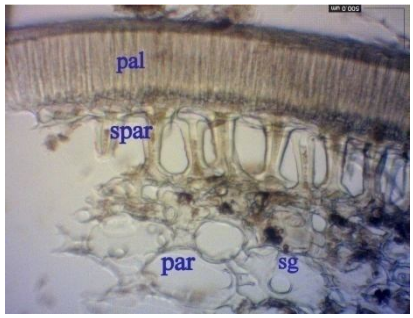


Fig. c

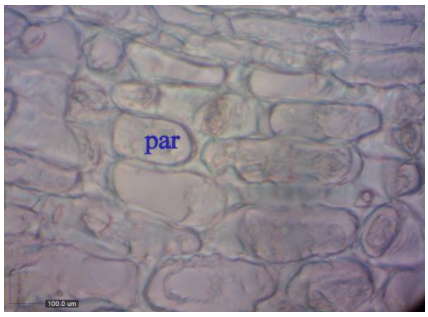


Fig. d

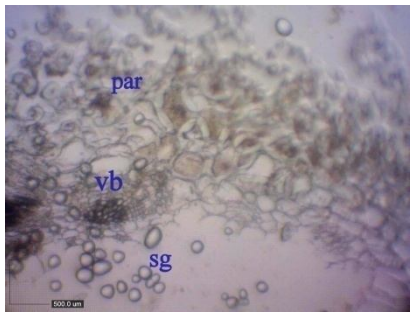


Fig. e

Fig a.: Detailed transverse section of seed passing through testa.
Fig b.: Enlarge view of palisade showing linea lucida.
Fig c.: Enlarge view of spool shaped parenchyma.
Fig d.: Enlarge view of testa showing parenchyma.
Fig e.: Enlarge view of testa showing parenchyma and traverse with vascular bundle.

Abbreviations-
cpar - collapsed parenchyma; ll - linea lucida; pal – palisade; par – parenchyma; sg - starch grains; spar - spool shaped parenchyma; vb - vascular bundle

Plate 9: Section passing through Cotyledon and Radical of *M. cochinchinensis* (White Seed)

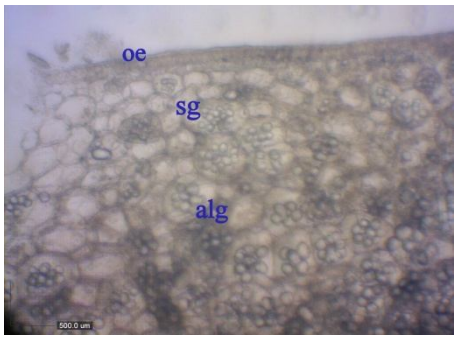


Fig. a

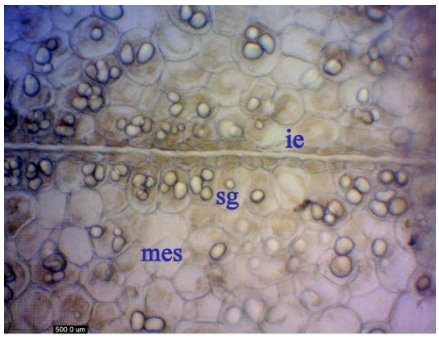


Fig. b

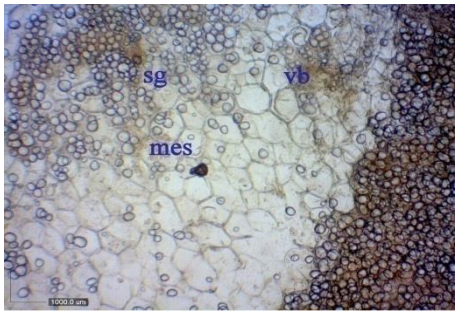


Fig. c

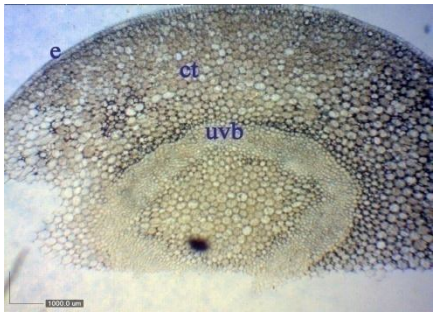


Fig. d

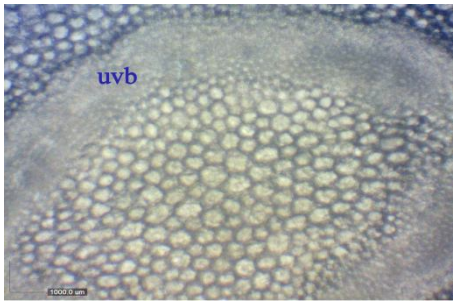


Fig. e

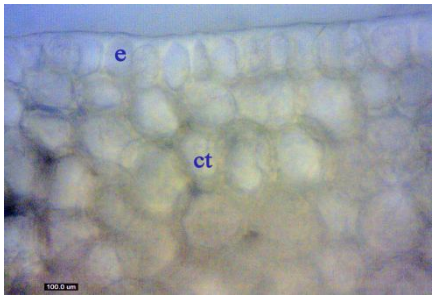


Fig. f

Fig a.: Section passing through cotyledon showing outer epidermis filled with starch grains and aleurone grains.

Fig b.: Section passing through cotyledon showing inner epidermis filled with starch grains and aleurone grains.

Fig c.: Section passing through cotyledon showing middle mesophyll cells filled with starch grains, aleurone grains and traverse with undeveloped vascular bundle.

Fig d.: Section passing through the radical.

Fig e.: Section passing through the radical showing undeveloped vascular bundle and pith region.

Fig f.: Enlarge view of cortex showing epidermis and cortex region.

Abbreviations-

alg -aleurone grains; ct – cortex; e – epidermis; ie - inner epidermis; meso – mesophyll; oe - outer epidermis; sg - starch grains; uvb - undeveloped vascular bundle; vb - vascular bundle.

Plate 10: Section passing through Hilum of *M. cochinchinensis* (White Seed)

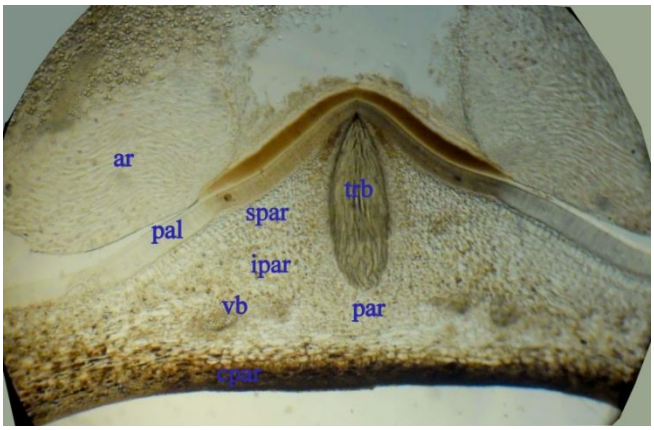


Fig. a

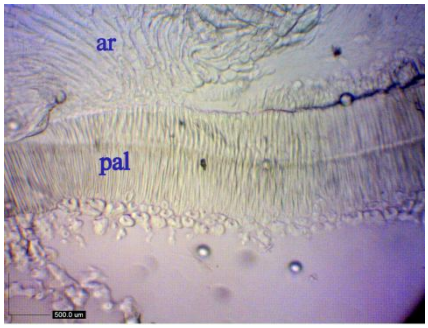


Fig. b

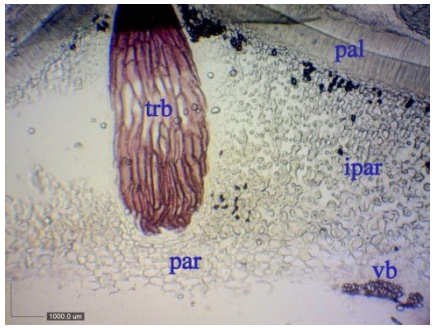


Fig. c

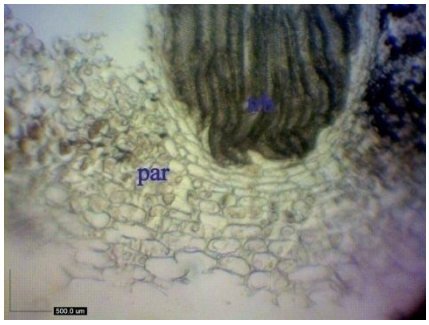


Fig. d

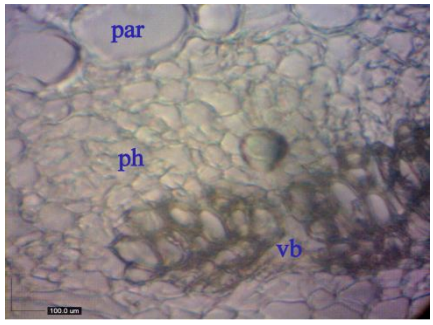


Fig. e

Fig a.: Detailed transverse section of seed passing through Hilum.
Fig b.: Enlarge view of section showing palisade cells.
Fig c.: Enlarge view of section showing tracheidal bar and irregular parenchyma.
Fig d.: Enlarge view of section showing tracheidal bar and lower parenchyma.
Fig e.: Enlarge view of section showing vascular bundle.

Abbreviations-
ar – arillus; cpar - collapsed parenchyma; ipar - irregular parenchyma; pal – palisade; par – parenchyma; ph – phloem; sg - starch grains; spar - spool shaped parenchyma; trb - tracheidal bar; vb - vascular bundle

Plate 11: Powder Characters of *M. cochinchinensis* (White Seed)

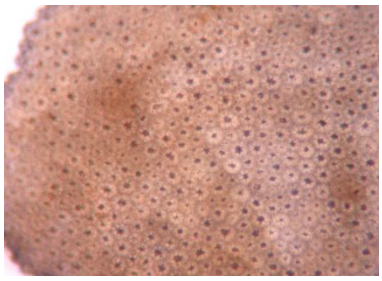


Fig.a

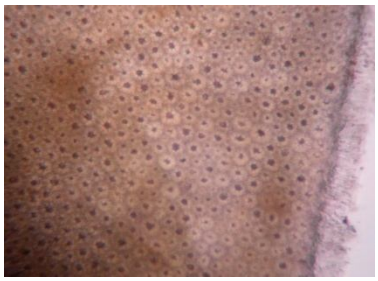


Fig.b



Fig.c

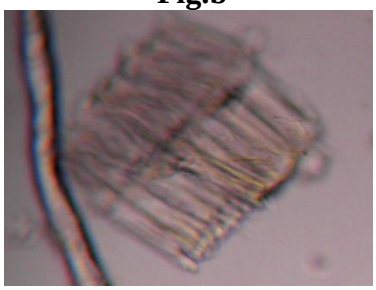


Fig.d



Fig.e



Fig.f

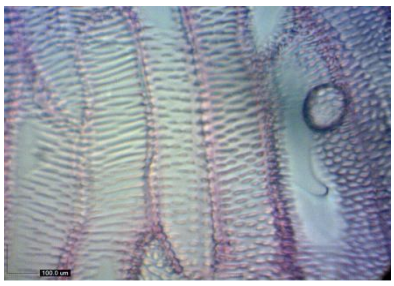


Fig.g



Fig.h

Fig a.: Fragment of palisade cells in surface view seen from above.
Fig b.: Fragment of palisade cells in surface view seen from below.
Fig c.: Fragment of palisade in sectional view from testa.
Fig d.: Fragment of double layered palisade in sectional view from hilum.
Fig e.: Isolated fragments of spool shaped cells in sectional view.
Fig f.: Fragment of spool shaped cells in surface view.
Fig g.: Fragment of trachedial bar.
Fig h.: Spiral vessels.

Plate 12: Powder Characters of *M. cochinchinensis* (White Seed)

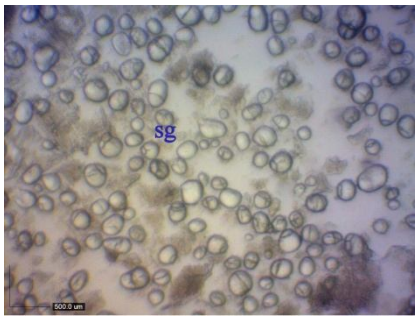


Fig. a



Fig. b

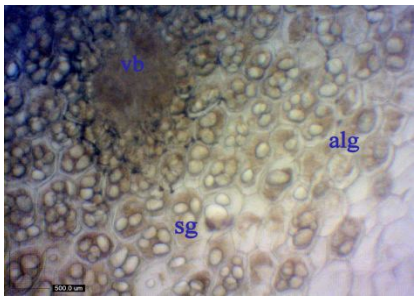


Fig. c

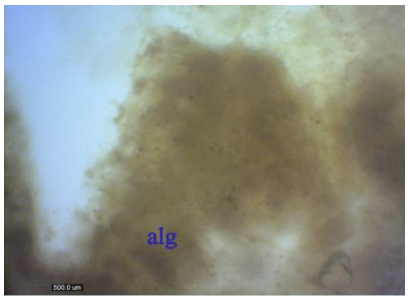


Fig. d



Fig. e

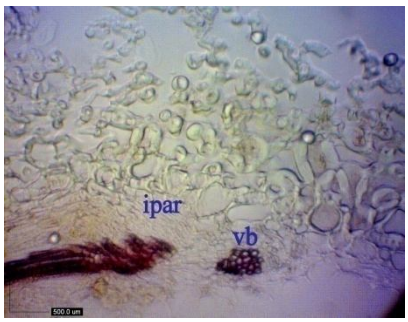


Fig. f

Fig a.: Isolated starch grain from cotyledon.
Fig b.: Isolated starch grains from radical.
Fig c.: Fragment of cotyledon showing aleurone grains and starch grains.
Fig d.: Fragment of radical showing aleurone grains and starch grains.
Fig e.: Isolated cells of arillus and starch grains.
Fig f.: Fragment of hilum showing irregular parenchyma traverse with vascular bundle.

Abbreviations-
alg -aleurone grains; ar – arrilus; ipar - irregular parenchyma; sg - starch grains; vb - vascular bundle

4.3 Pharmacognostic evaluation of *Mucuna cochinchinensis* (Lour.) A. Chev. (Black Seed)

4.3.1 Macroscopy

Seeds are ovate, oblong, globose, 1.25 to 2.19 cm in length, 0.93 to 1.5 cm in breadth and 0.45 to 1.16 cm in thickness. Surface hard, smooth, glossy, brown to black. Hilum conspicuous, 0.6 to 0.8 cm long, more or less elongated with indistinct slit, cushioned with white spongy tissue and bordered with aril located at one of the longer edge of seed near micropylar end.

4.3.2 Microscopy

Diagrammatically T. S. of seed is oval and L.S. is oblong to oval in shaped.

Testa: Detailed T.S. passing through centre of seed shows the outermost malpighian layer of testa composed of thick walled, cylindrical palisade cells, with narrow lumen broadest at base, often filled with brown colouring matter, through which a light line linea lucida extend across, covered with thin cuticle, underneath this lies a layer of sub epidermis consisting of spool or bone shaped cells with broad apical and basal ends connecting with long vertically running narrow strips, followed by 9 to 15 rows of spongy parenchyma with somewhat thickened walls, the cells of the outer region are big in size with large intercellular spaces unlike the cells of the middle region which are smaller in size, with few intercellular spaces, embedded with vascular strands and the cells of the innermost tissue being obliterated.

Cotyledon: consists of upper and lower epidermis embedded with aleurone grains; enclosing the wide zone of mesophyll containing starch grains, aleurone grains. Mesophyll cells traverse with developed and undeveloped vascular bundles.

Hilum: Section passing through the hilum differ from the above in following respect: It shows two rows of palisade cells underneath the spongy parenchyma of hilum cushion, lying adjacent to the arillus, sub epidermis forms a thick, loose tissue of variously shaped cells with dark contents, embedded with tracheidal bar and recurrent vascular bundles, a narrow groove run from the centre of the two rows of palisade cells, connecting the tissue of the tracheidal bar.

Plate 13: Morphology of *Mucuna cochinchinensis* (Lour.) A. Chev. (Black Seed)



Habit



Flowering twig



Fruiting twig



Dry pod



Dry Seeds



Seed Powder

4.3.3 Powder Microscopy (Organoliptic characters)

Seed powder is creamish gray in colour, coarse, odourless, and taste starchy and slightly bitter. Seed powder shows the characters like

1. Fragment of Palisade cells in surface view seen from above.
2. Fragment of Palisade cells in surface view seen from below.
3. Fragment of Palisade in sectional view from testa.
4. Fragment of double layered Palisade in sectional view from Hilum region.
5. Isolated fragments of spool shaped cells in sectional view.
6. Fragment of spool shaped cells in surface view.
7. Fragment of tracheidal bar.
8. Spiral vessels
9. Isolated starch grain from cotyledon.
10. Isolated starch grains from radical.
11. Fragment of cotyledon showing aleurone grains and starch grains.
12. Fragment of radical showing aleurone grains and starch grains.
13. Isolated cells of Arillus.
14. Fragment of irregular parenchyma from hilum region.

Plate 14: Section passing through Testa of *M. cochinchinensis* (Black seed)

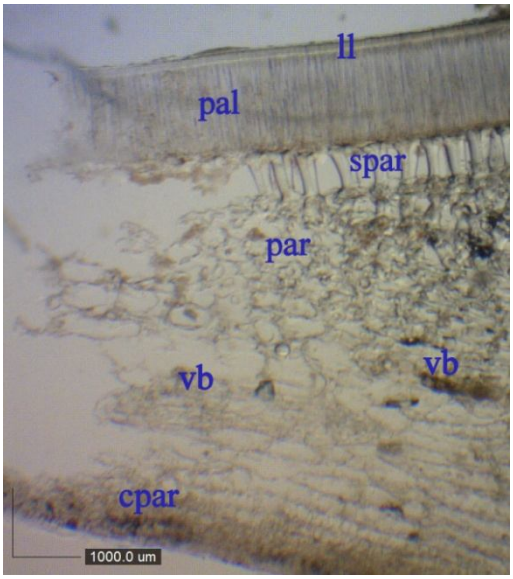


Fig. a

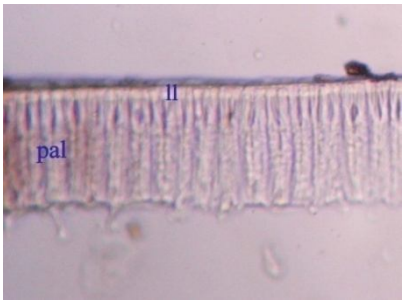


Fig. b

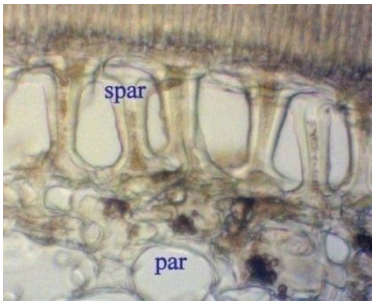


Fig. c

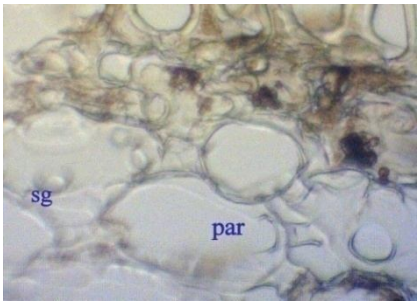


Fig. d



Fig. e

Fig a.: Detailed transverse section of seed passing through testa.
Fig b.: Enlarge view of palisade showing linea lucida.
Fig c.: Enlarge view of spool shaped parenchyma.
Fig d.: Enlarge view of testa showing parenchyma.
Fig e.: Enlarge view of testa showing parenchyma and traverse with vascular bundle.

Abbreviations-
cpar - collapsed parenchyma; ll - linea lucida; pal - palisade; par - parenchyma; sg - starch grains; spar - spool shaped parenchyma ; vb - vascular bundle

Plate 15: Section passing through Cotyledon and Radical of *M. cochinchinensis* (black seed)

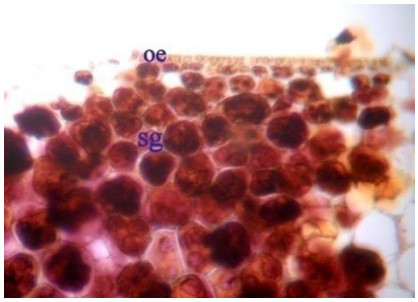


Fig. a

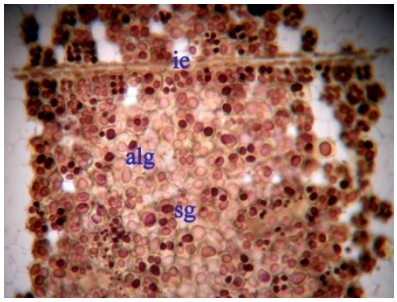


Fig. b

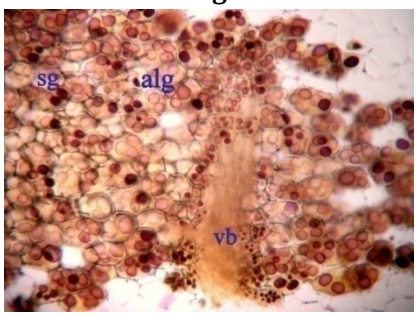


Fig. c

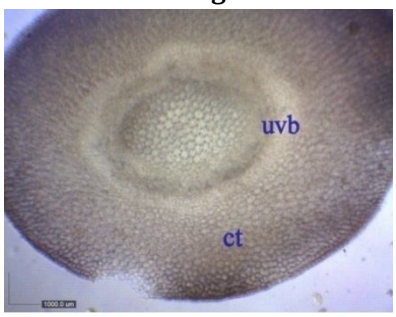


Fig. d

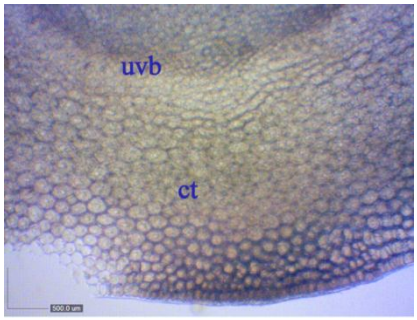


Fig. e



Fig. f

Fig a.: Section passing through cotyledon showing outer epidermis filled with starch grains and aleurone grains.

Fig b.: Section passing through cotyledon showing inner epidermis filled with starch grains and aleurone grains.

Fig c.: Section passing through cotyledon showing middle mesophyll cells filled with starch grains, aleurone grains and traverse with undeveloped vascular bundle.

Fig d.: Section passing through the radical.

Fig e.: Section passing through the radical showing epidermis and cortex region.

Fig f.: Enlarge view of cortex.

Abbreviations-

alg -aleurone grains; ct - cortex; e - epidermis; ie - inner epidermis; oe - outer epidermis; sg - starch grains; uvb - undeveloped vascular bundle; vb - vascular bundle.

Plate 16: Section passing through Hilum of *M. cochinchinensis* (Black seed)

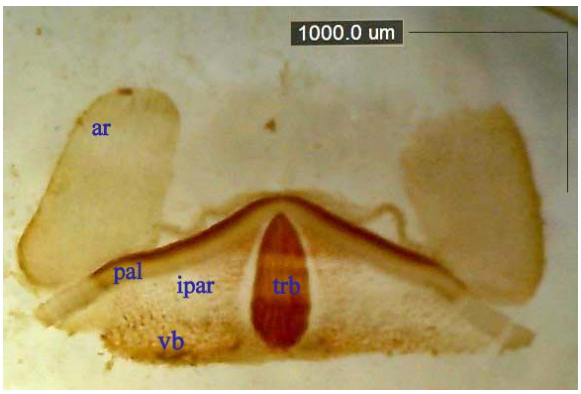


Fig. a

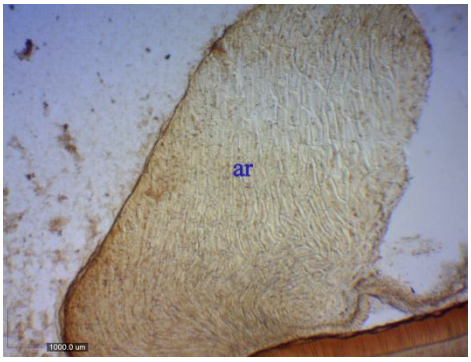


Fig. b

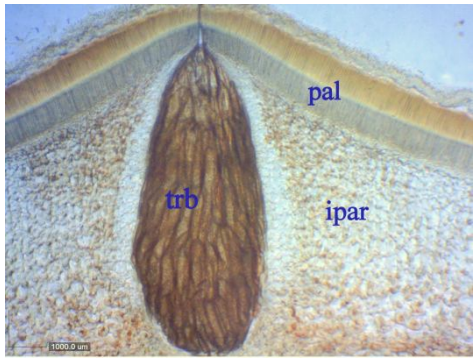


Fig. c

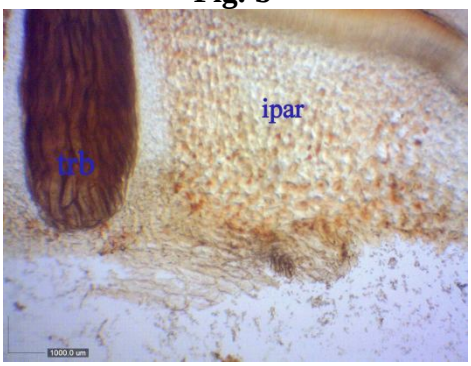


Fig. d

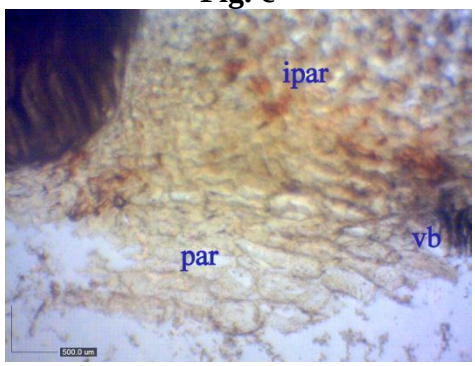


Fig. e

Fig a.: Detailed transverse section of seed passing through Hilum.
Fig b.: Enlarge view of section showing arillus.
Fig c.: Enlarge view of section showing tracheidal bar and irregular parenchyma.
Fig d.: Enlarge view of section showing tracheidal bar and lower parenchyma.
Fig e.: Enlarge view of section showing vascular bundle.

Abbreviations-
ar – arillus; ipar - irregular parenchyma; pal – palisade; par – parenchyma; trb - tracheidal bar
vb - vascular bundle

Plate 17: Powder Characters of *M. cochinchinensis* (Black Seed)

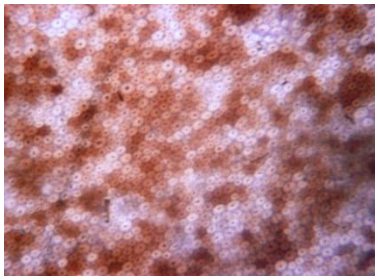


Fig.a

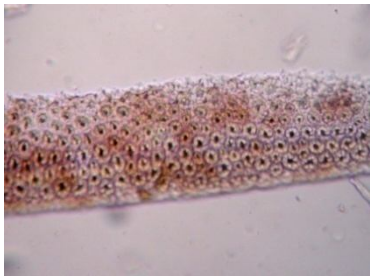


Fig.b

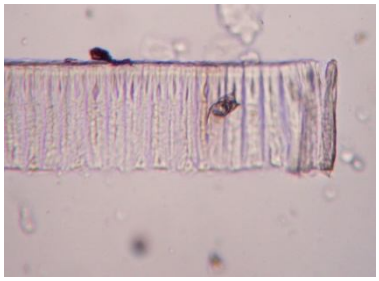


Fig.c

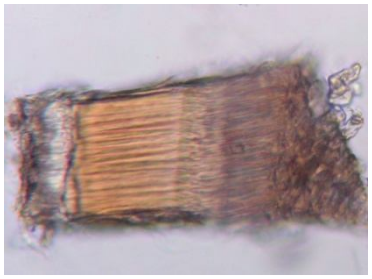


Fig.d



Fig.e

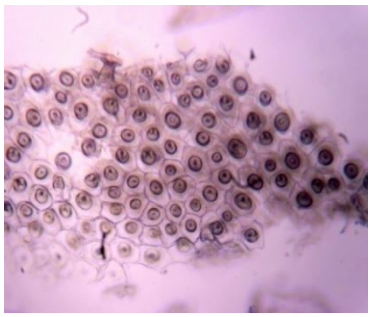


Fig.f

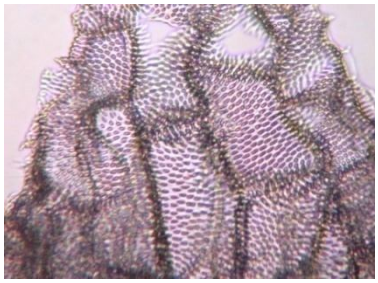


Fig.g

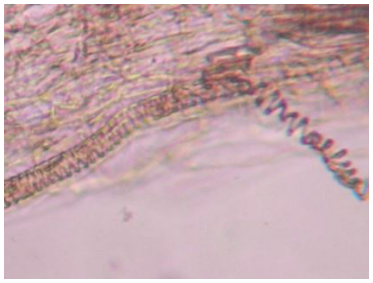


Fig.h

Fig a.: Fragment of palisade cells in surface view seen from above.
Fig b.: Fragment of palisade cells in surface view seen from below.
Fig c.: Fragment of palisade in sectional view from testa.
Fig d.: Fragment of double layered palisade in sectional view from hilum.
Fig e.: Isolated fragments of spool shaped cells in sectional view.
Fig f.: Fragment of spool shaped cells in surface view.
Fig g.: Fragment of tracheidal bar.
Fig h.: Spiral vessels.

Plate 18: Powder Characters of *M. cochinchinenssis* (Black Seed)

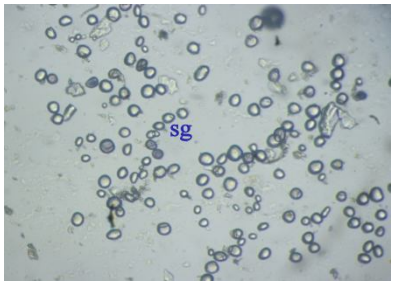


Fig. a

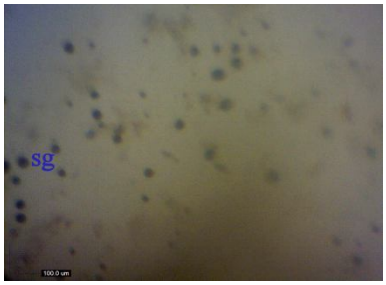


Fig. b

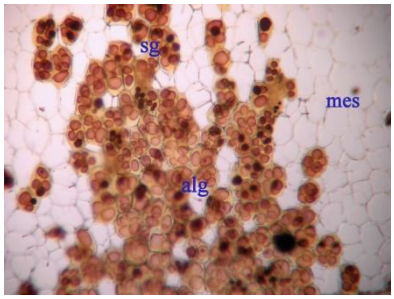


Fig. c

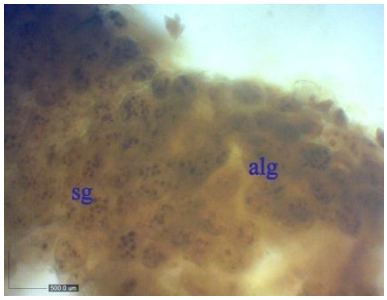


Fig. d



Fig. e

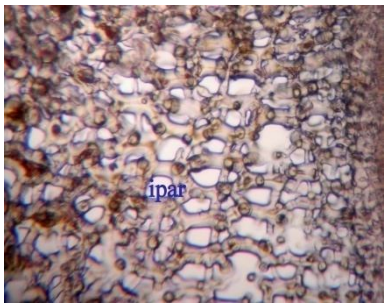


Fig. f

Fig a.: Isolated starch grain from cotyledon.
Fig b.: Isolated starch grains from radical.
Fig c.: Fragment of cotyledon showing aleurone grains and starch grains.
Fig d.: Fragment of radical showing aleurone grains and starch grains.
Fig e.: Isolated cells of arillus and starch grains.
Fig f.: Fragment of hilum showing irregular parenchyma.

Abbreviations :
alg -aleurone grains; sg - starch grains; mes - mesophyll cell; ar – arillus; ipar - irregular parenchyma

4.4 Comparative account of *Mucuna* sps:

Comparative account of *Mucuna* sps using morphological, macro and microscopic characters of seeds are depicted in Table 4.1 - 4.3 and Plate 19-23.

Table 4.1: Comparative morphological characters of *Mucuna* species

Morphological characters	*M. cochinchinensis* (Black)	*M. cochinchinensis* (White)	*M. pruriens*
Habit	Annual twiner	Annual twiner	Annual twiner
Flower	white or pale purple	Dark purple	purple
Pod	10-15 cm long, 1-2.5 cm wide, green with black bristles when young, black, wrinkled and destitude of down when ripe, bristles not irritant	10-15 cm long, 1-2.5 cm wide, green with white bristles when young, black, wrinkled and destitutde of down when ripe, bristles not irritant	5-10 cm long, 1.5-1.8 cm wide, light green with white bristles when young, pale brown or gray, longitudinally ribbed, turgid, densely bristles persistant, irritant.
Seed	Brown to black, 6-8 in a pod.	Dull white, 6-8 in a pod.	Black, 4-6 in a pod.

Table 4.2: Comparative macroscopic characters of seeds of *Mucuna* species

Macroscopic characters	*M. cochinchinensis* (Black)	*M. cochinchinensis* (White)	*M. pruriens*
Shape	ovate, oblong, globose	ovate, oblong, globose	ovate, oblong, flattened
Size	length -1.25 - 2.19 cm, breadth- 0.93 - 1.5 cm, thickness -0.45 - 1.16 cm.	length -1.20 - 2, breadth- 0.95 -1.37 cm thickness -0.50 - 1 cm	length -0.8 -1.29 cm, breadth- 0.6 to 0.9 cm, thickness -0.3 - 0.63 cm
Surface	hard, smooth, glossy	hard, smooth, glossy	hard, smooth, glossy
Colour	brown to black	dull white	brown to black
Hilum	conspicuous, 0.6 - 0.8 cm long	Conspicuous, 0.7 - 0.9 cm long.	Conspicuous 0.4 - 0.5 cm long.
Powder characters			
Colour	creamish gray	yellowish cream	creamish gray
Texture	course	course	course
Odour	odourless	odourless	odourless
Taste	starchy - slightly bitter	starchy -slightly bitter	starchy - slightly bitter

Table 4.3: Comparative microscopic characters of *Mucuna* species

Microscopic characters	***M. cochinchinensis* (Black)**	***M. cochinchinensis* (White)**	***M. pruriens***
Palisade cells	Lumen narrow and broadest at apical end	Lumen narrow and broadest at apical end and base	Lumen narrow and broadest at base
Spongy parenchyma	No. of layers 18-20 outer 7-8 layers small with few inter cellular spaces Middle 8-9 layers elongated cells with compact arrangement inner 3-4 collapsed layers	No. of layers 14-16 outer 7-8 layers big with inter cellular spaces Middle 5-6 layers with few inter cellular spaces inner 3-4 collapsed layers	No. of layers 9-16 outer 7-8 layers small, compact middle 5-6 layers elongated, compact inner 3-4 collapsed layers
No. of mesophill layers in cotyledon	up to 30	up to 35	up to 20

Plate 19: Comparative morphological characters of *Mucuna* species

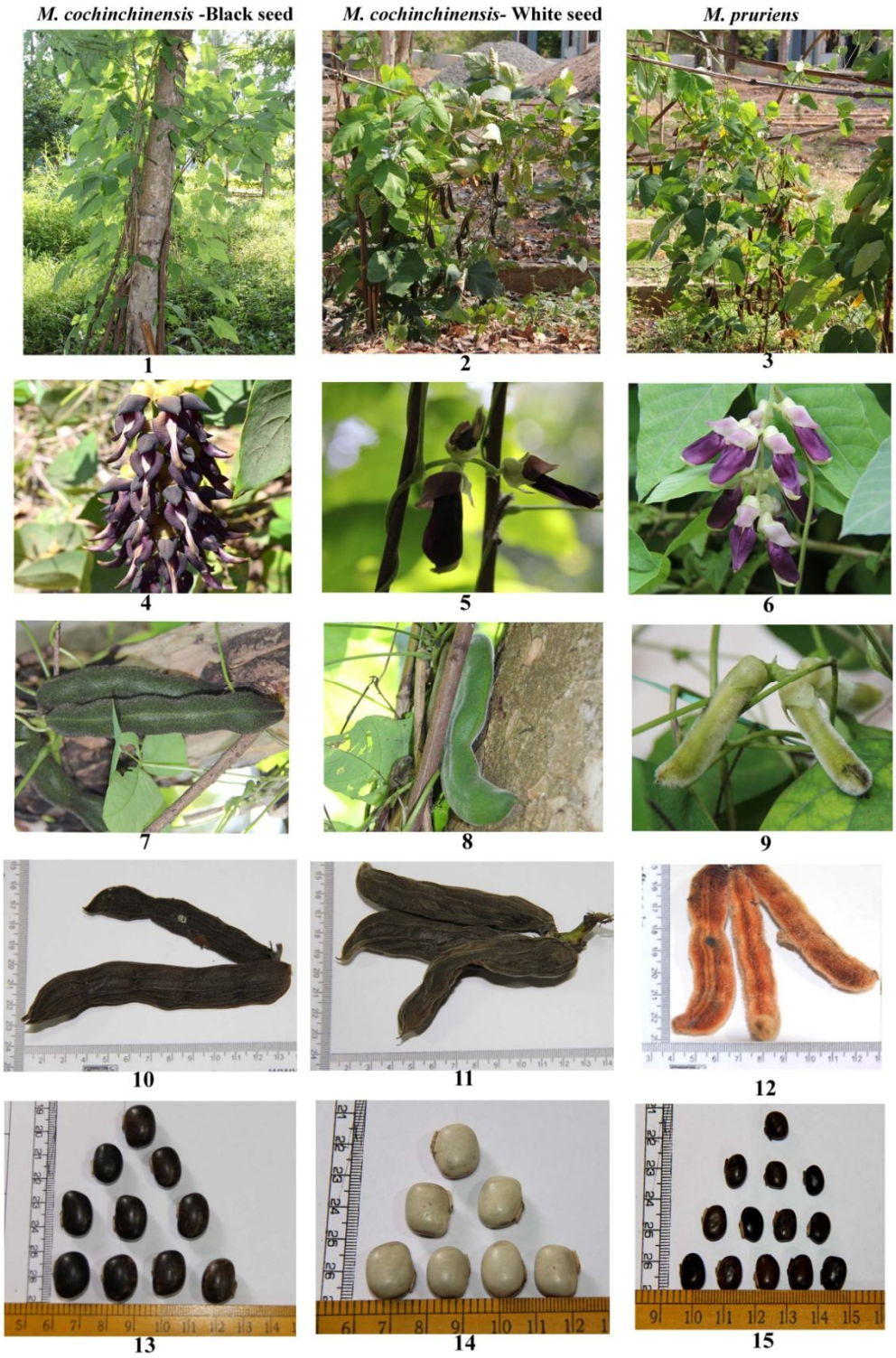


Figure 1: 1,2,3- Habit; 4,5,6- Flowers; 7,8,9- Fresh pods; 10,11,12- Mature dried pods; 13,14,15- Mature dried seeds

Plate 20: Comparative microscopic characters of Seeds

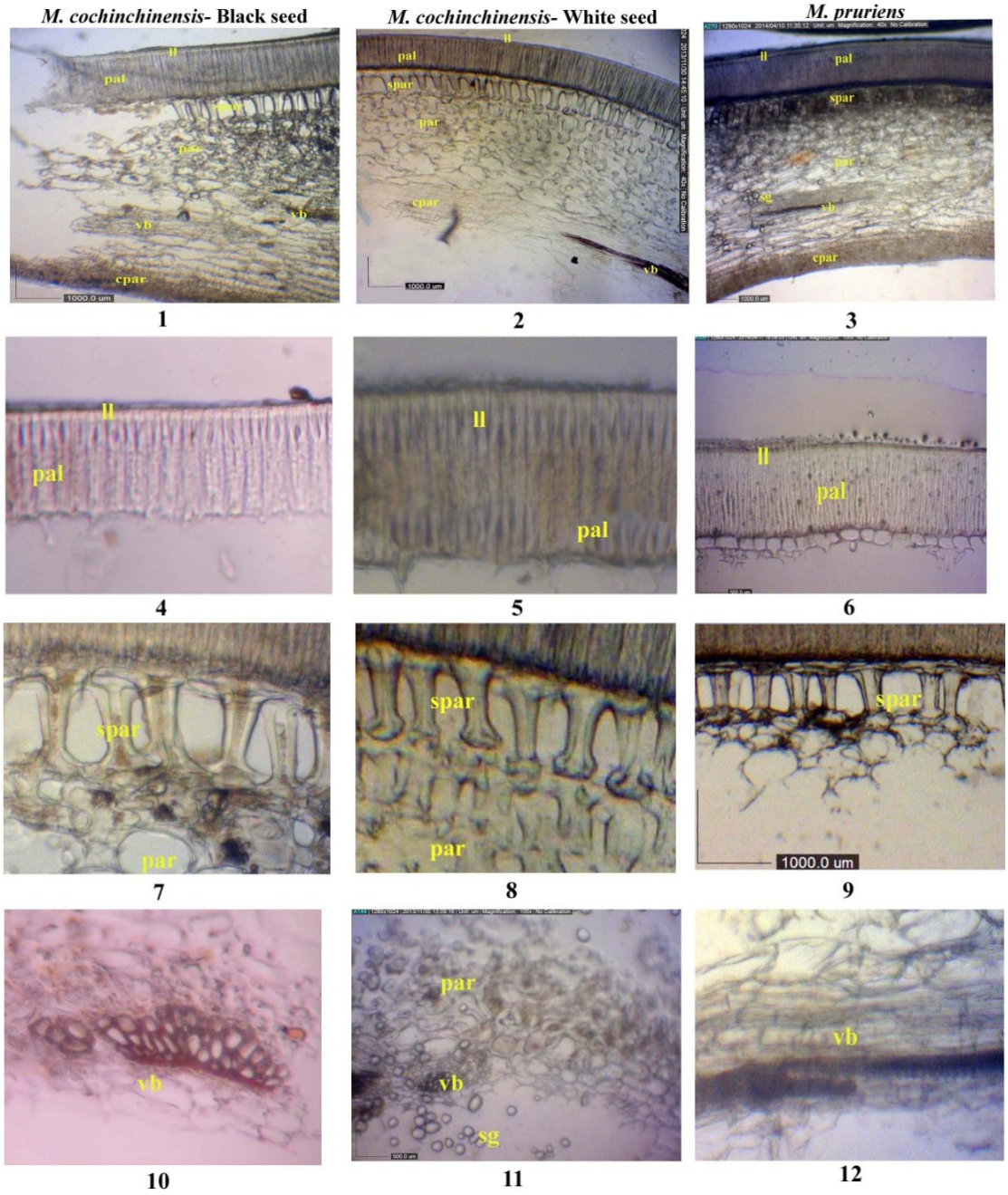


Figure 2: 1,2,3- Transverse section of seed passing through testa; 4,5,6- Enlarge view of palisade showing linea lucida; 7,8,9- Enlarge view of spool shaped parenchyma; 10,11,12- Enlarge view of testa showing parenchyma traverse with vascular bundle.

Plate 21: Comparative Transverse Section of cotyledon

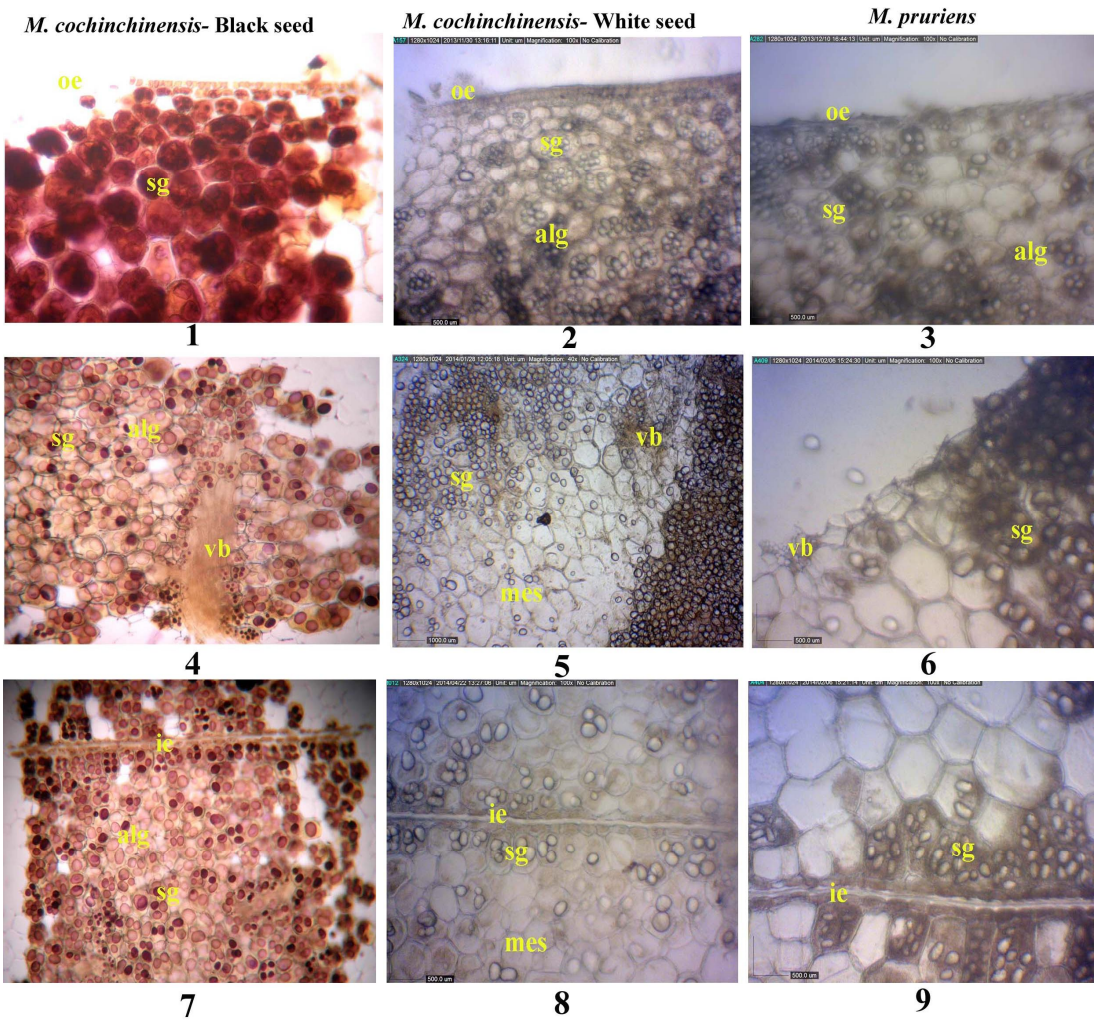


Figure 3: 1,2,3- TS of cotyledon showing outer epidermis; 4,5,6- TS of cotyledon showing middle mesophyll cells; 7,8,9- TS of cotyledon showing inner epidermis.

Plate 22: Transverse section of seed passing through hilum

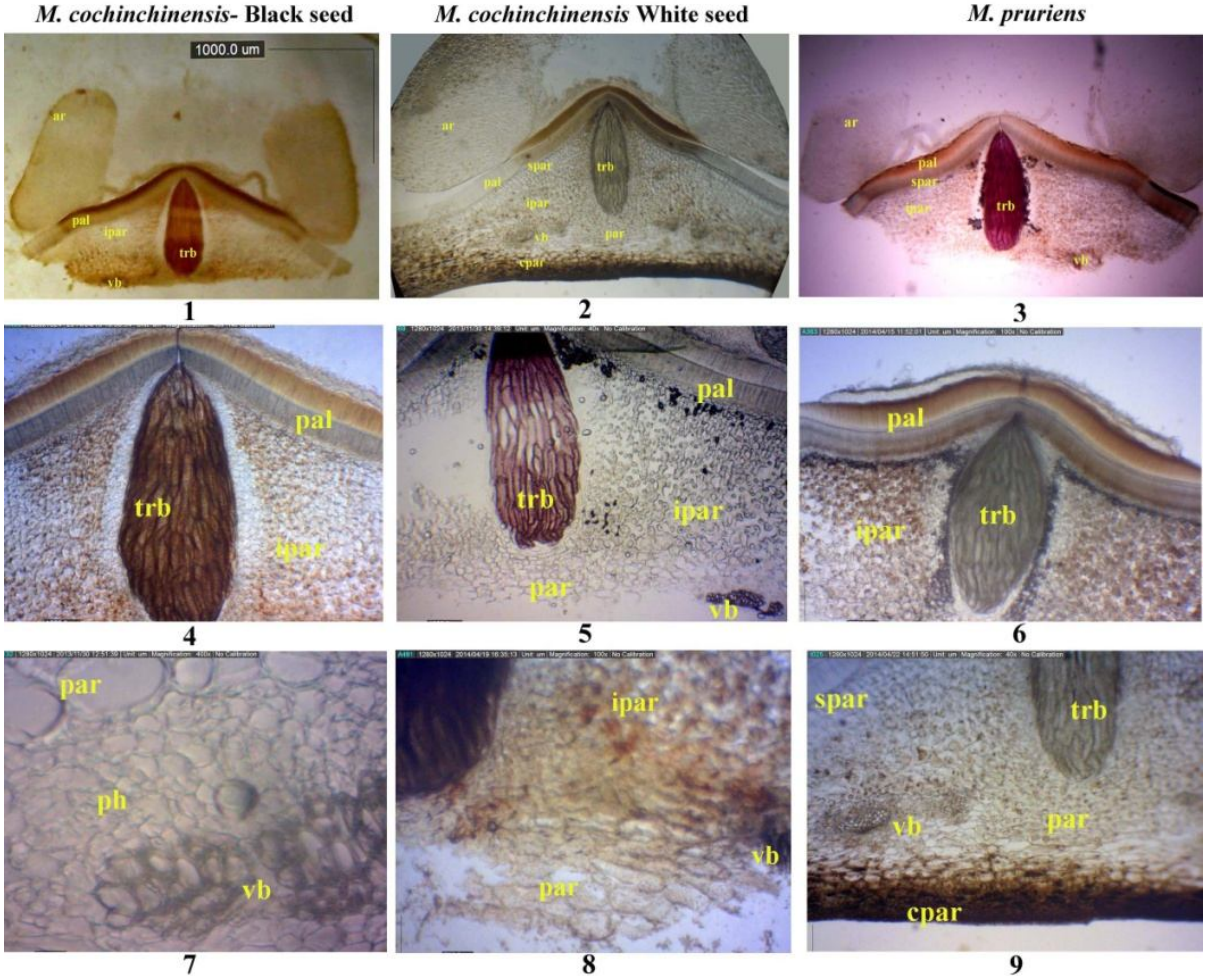


Figure 4:1,2,3-Transverse section of seed passing through hilum; 4,5,6- Enlarge view showing double layered palisade, tracheidial bar and irregular parenchyma; 7,8,9- Enlarge view showing lower parenchyma and vascular bundle.

Plate 23a: Comparative characteristics of *Mucuna* seed powder

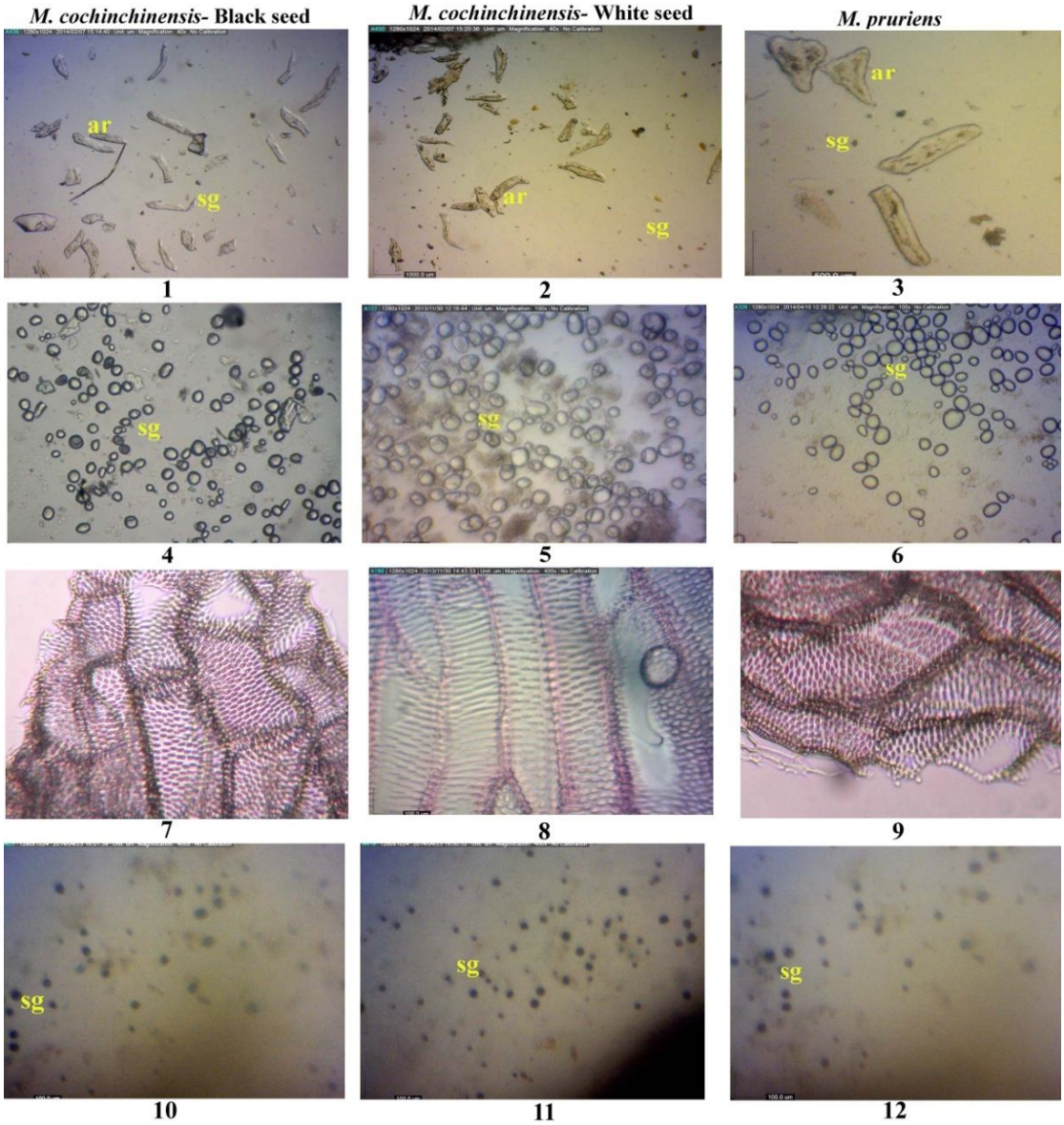


Figure 5: 1,2,3- Isolated cells of arrilus; 4,5,6- Isolated starch grains from cotyledon; 7,8,9- Fragment of tracheidal bar; 10,11,12- Isolated starch grains from radical.

Plate 23b: Comparative characteristics of *Mucuna* seed powder

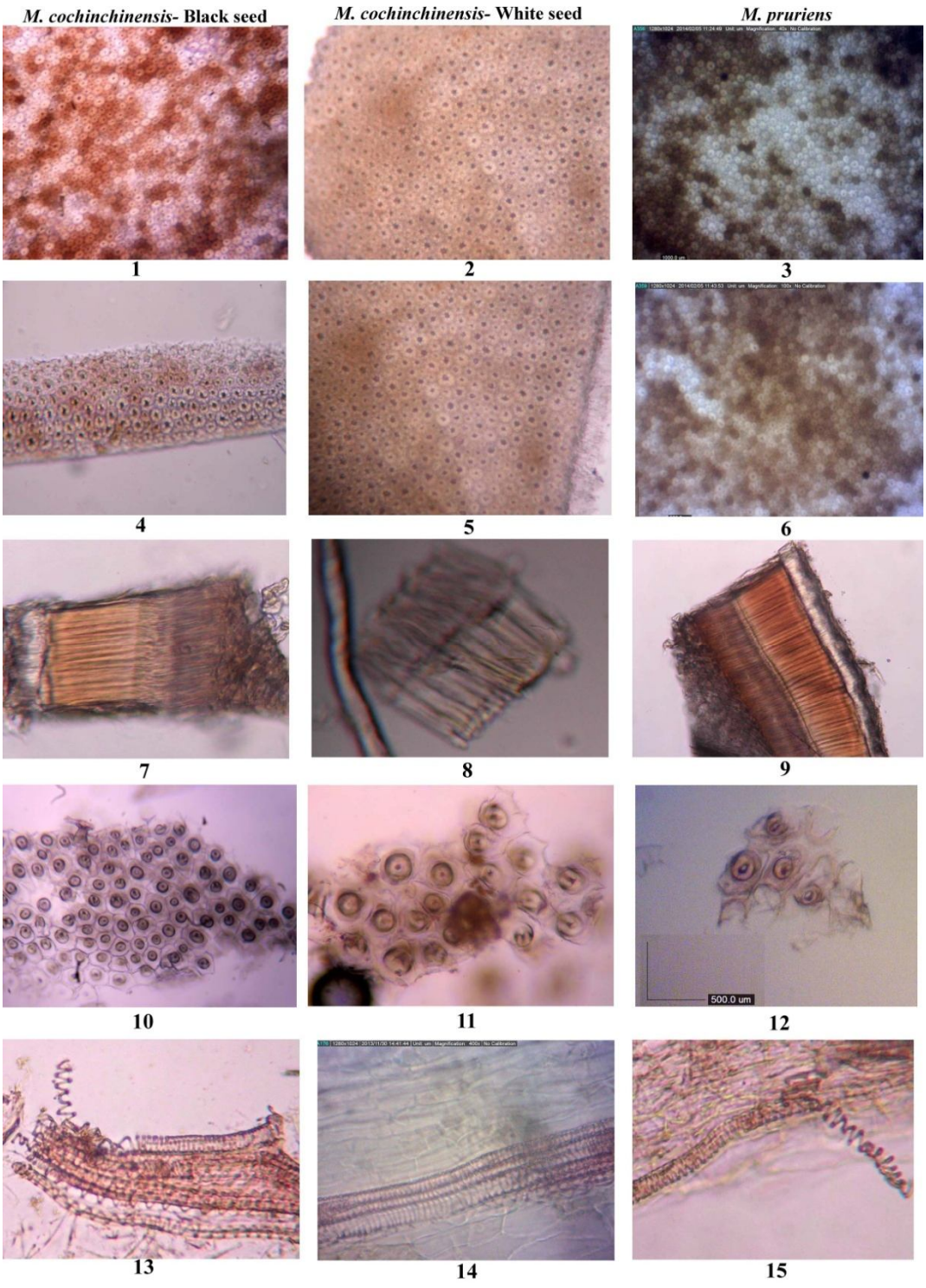


Figure 6: 1,2,3- Palisade cells in surface view seen from above; 4,5,6- Palisade cells in surface view seen from below; 7,8,9- 2 layers of palisade cells from the hilum region; 10,11,12- Spool shaped cells in surface view; 13,14,15- Spiral vessels.

B. Phytochemistry of *Mucuna* sps.

4.5 Physico - chemical analysis

Seed Powder *M. pruriens* (L.) DC., *M. cochinchinensis* (Lour.) A. Chev white and black seed showed total ash 3.88%, 3.42%, 3.53% ; Acid insoluble ash 0.09%, 0.04%, 0.10%; Water soluble ash 0.14%, 0.05%, 0.07%; LOD 5.56%, 10.15%, 9.29%. Aqueous solution of seeds were alkaline in nature and showed pH 7.59, 7.43, 7.40 respectively (Table 4.4).

Table 4.4: Physico- chemical analysis

Sr. No.	Parameters	*Mucuna pruriens* (L.) DC.	*Mucuna cochinchinensis* (Lour.) A. Chev. (White Seed)	*Mucuna cochinchinensis* (Lour.) A. Chev. (Black Seed)
1	Foreign matter	2.02%	0.08%	1.90%
2	Total Ash	3.88%	3.42%	3.53%
3	Acid Insoluble Ash	0.09%	0.04%	0.10%
4	Water soluble Ash	0.14%	0.05%	0.07%
5	Loss on Drying (LOD)	5.56%	10.15%	9.29%
6	pH	7.59	7.43	7.40
7	Swelling factor	0.0 ml	1.0 ml	1.0 ml

4.6 Extractive Values

Extractive values of *M. pruriens, M. cochinchinensis* white and black seeds in different solvents such as, water soluble extractive 29%, 21.89%, 24.82%; Absolute alcohol soluble extractives 9.61%, 13.46%, 14.08%; Petroleum ether soluble extractives 5.34%, 5.17%, 11.41%; Chloroform soluble extractive 8.20%, 8.22%, 15.05%; Carbon tetra chloride soluble extractives 8.20%, 7.61%, 11.12%; Ethyl acetate soluble extractives 5.02%, 5.17%, 9.60%; Acetone soluble extractive 7.41%, 6.07%, 10.74%; methanol soluble extractives 19.05%, 12.32%, 15.83%; Benzene soluble extractive 5.84%, 5.63%, 11.05% and in Hydro-alcoholic soluble extractives 38.64%, 38.13%, 38.05% respectively (Table 4.5).

Table 4.5: Determination of extractive Values

Sr. No.	Extract	Extractive values		
		M. pruriens	***M. cochinchinenesis* (White)**	***M. cochinchinensis* (Black)**
1	Absolute Alcohol	9.61%	13.46%	14.08%
2	Water	29.00%	21.89%	24.82%
3	Methanol	19.05%	12.32%	15.83%
4	Acetone	7.41%	6.07%	10.74%
5	Ethyl acetate	5.02%	5.17%	9.60%
6	Petroleum ether	5.34%	5.17%	11.41%
7	Benzene	5.84%	5.63%	11.05%
8	Chloroform	8.20%	8.22%	15.05%
9	Carbon tetra chloride	8.20%	7.61%	11.12%
10	Hydro-alcoholic (50:50%)	38.64%	38.13%	38.05%

4.7 Fluorescence analysis of *Mucuna* sps

The results of fluorescence analysis of *Mucuna* seeds with different solvents are expressed in Table 4.6. Fluorescence study is an essential and required parameter for first line standardization of crude drug. In this analysis most of the reactions resulted in colourless reaction mixture.

Table 4.6: Fluorescence of *Mucuna* species with different solvents

Sr. No.	Solvents	***Mucuna pruriens* (L.) DC.**			***Mucuna cochinchinensis* (Lour.) A. Chev. (White Seed)**			***Mucuna cochinchinensis* (Lour.) A. Chev. (Black Seed)**		
		Day light	**Short UV**	**Long UV**	**Day light**	**Short UV**	**Long UV**	**Day light**	**Short UV**	**Long UV**
1	Water	Light brown	Light umber	Light purple	Dark brown	Dark blackish green	Dark purple	Light brown	Chestnut	Dark purple
2	Acetone	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless
3	Benzene	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless
4	Chloroform	Colourless	Colourless	Colourless	Light yellow	Colourless	Colourless	Colourless	Buff	Colourless
5	Carbon tetra chloride	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Very Light brown	Light yellow	Colourless

6	Ethanol	Light yellow	Colour less	Colour less	Colour less	Colourless	Colour less	Light yellow	Light yellow	Colour less
7	Ethyl acetate	Colour less	Colour less	Colour less	Colour less	Colourless	Colour less	Light yellow	Light yellow	Colour less
8	Methanol	Light yellow	Colour less	Colour less	Light pale yellow	Light pale yellow	Colour less	Light yellow	Light yellow	Colour less
9	Petroleum ether	Colour less	Colour less	Colour less	Colour less	Colourless	Colour less	Light yellow	Light yellow	Colour less

Table 4.7: Dimensional parameters (length, breadth and width) of seeds

Species Sr. No.	***Mucuna pruriens* (L.) DC.** (cm)			***Mucuna cochinchinensis* (Lour.) A. Chev. (White Seed)** (cm)			***Mucuna cochinchinensis* (Lour.) A. Chev. (Black Seed)** (cm)		
	L	**B**	**W**	**L**	**B**	**W**	**L**	**B**	**W**
1	1.16	0.82	0.60	2.00	1.24	0.90	1.90	1.40	1.0
2	1.20	0.89	0.56	1.90	1.30	0.87	2.12	1.49	1.11
3	1.20	0.85	0.55	1.55	1.40	0.80	1.95	1.39	0.95
4	1.23	0.90	0.63	1.82	1.34	0.99	2.19	1.44	1.16
5	1.29	0.90	0.55	1.80	1.20	0.95	2.13	1.39	1.05
6	1.15	0.80	0.54	1.85	1.37	1.00	2.03	1.46	1.14
7	1.22	0.91	0.60	1.54	1.30	0.96	2.06	1.50	1.06
8	1.22	0.80	0.54	1.60	1.24	0.64	2.04	1.43	1.09
9	1.29	0.85	0.55	1.54	1.20	0.90	2.04	1.34	0.95
10	1.29	0.84	0.53	1.60	1.15	0.90	2.04	1.36	0.81
11	1.10	0.82	0.55	1.62	1.19	0.80	1.72	1.23	0.80
12	1.12	0.81	0.54	1.45	1.10	0.85	1.90	1.40	1.03
13	1.23	0.75	0.52	1.54	1.14	0.89	2.00	1.49	0.90
14	0.89	0.78	0.57	1.30	1.00	0.70	1.65	1.20	0.89
15	1.05	0.80	0.54	1.74	1.22	0.80	1.59	1.19	0.85
16	1.15	0.82	0.56	1.50	1.16	0.75	1.80	1.20	0.79
17	0.90	0.69	0.44	1.42	1.06	0.82	2.13	1.41	0.83
18	1.02	0.64	0.50	1.53	1.10	0.83	2.00	1.40	0.80
19	0.95	0.79	0.52	1.50	1.06	0.80	1.40	1.14	0.70
20	1.0	0.73	0.45	1.56	1.19	0.87	1.50	1.10	0.63
21	0.84	0.60	0.52	1.50	1.03	0.73	1.53	1.10	0.74
22	0.95	0.75	0.32	1.39	1.00	0.72	1.41	1.10	0.84
23	0.92	0.60	0.45	1.20	1.03	0.59	1.50	1.10	0.84
24	0.90	0.70	0.45	1.25	1.00	0.52	1.32	0.95	0.45
25	0.80	0.63	0.30	1.28	0.95	0.50	1.25	0.93	0.59
Result									
Minimum	0.80	0.60	0.30	1.20	0.95	0.50	1.25	0.93	0.45
Maximum	1.29	0.91	0.63	2.00	1.37	1.00	2.19	1.50	1.16
Average	1.08	0.78	0.52	1.56	1.16	0.80	1.81	1.29	0.88

***** **Abbreviations-: L**- Length, **B**- Breadth, **W**- Width

****** All above measurements are in centimeters.

Table 4.8: Weight variation of seeds (total no. of weighings-100) of *Mucuna* sps

Sr. No.	*M. pruriens* (g)	*M. cochinchinensis* (white Seed) (g)	*M. cochinchinensis* (Black Seed) (g)
1	0.0484	1.3304	1.2525
2	0.3086	1.1540	1.1503
3	0.3505	1.1645	1.5330
4	0.3352	1.6275	1.4610
5	0.4336	1.2214	1.3907
6	0.3778	1.1833	1.1083
7	0.3221	0.7727	2.3017
8	0.3915	0.7985	1.3902
9	0.3360	1.1218	1.4852
10	0.2370	0.5952	1.3929
11	0.3625	1.0360	1.8909
12	0.4651	0.8939	2.4259
13	0.2814	1.3599	1.5007
14	0.4545	1.5601	2.2990
15	0.3912	1.8614	1.9371
16	0.3268	0.9282	1.7440
17	0.3145	1.1898	1.9502
18	0.3915	0.9012	1.6494
19	0.4847	0.8142	1.6995
20	0.4640	1.4490	1.9955
21	0.2236	1.6370	1.7634
22	0.5256	1.2555	1.8884
23	0.4719	1.3578	1.4898
24	0.4889	1.2718	1.2002
25	0.3048	1.0207	1.6743
26	0.4085	1.1288	1.8782
27	0.3395	0.9317	2.0380
28	0.4374	1.4919	1.7664
29	0.3497	0.9043	2.0509
30	0.2955	0.4314	2.1554
31	0.3217	1.7847	1.8174
32	0.3134	1.1871	1.8133
33	0.3724	1.3095	1.4073
34	0.4569	1.4227	2.0794
35	0.2158	1.5152	2.1059
36	0.3553	1.3980	1.2358
37	0.4162	1.3708	1.8317
38	0.3880	1.3212	1.9873
39	0.3445	0.9151	1.6091
40	0.4742	0.9505	2.5052
41	0.4788	1.3560	2.5804
42	0.2845	1.1415	1.4409
43	0.3824	0.8504	1.6041
44	0.3734	1.2571	1.5951
45	0.5027	0.9718	1.1759
46	0.3136	0.6177	2.0074
47	0.3168	1.4147	1.2499
48	0.3638	1.7699	1.1734
49	0.4278	1.2656	1.2844
50	0.2646	1.5088	1.0899
51	0.1699	1.2824	2.2988
52	0.3690	0.9525	1.2132
53	0.4280	1.2912	1.9735

54	0.3375	0.5880	1.6232
55	0.3866	1.0340	1.0692
56	0.3712	0.5115	1.8332
57	0.3156	1.3618	2.0113
58	0.2695	1.1673	2.4177
59	0.4364	1.7213	1.4170
60	0.3990	1.1661	1.8586
61	0.3357	0.7748	1.0454
62	0.4505	0.8760	1.8644
63	0.3181	0.9975	1.6823
64	0.3844	0.8322	2.6697
65	0.4089	1.1799	1.3120
66	0.4940	1.5565	1.2718
67	0.3086	1.3955	1.3370
68	0.3298	1.3436	1.2700
69	0.2427	1.6960	1.0490
70	0.2532	1.2442	1.4264
71	0.2705	1.0059	1.1671
72	0.2410	1.0940	1.1531
73	0.2377	1.0157	1.9039
74	0.2032	1.3828	1.5192
75	0.1728	1.4269	1.5336
76	0.1992	1.4280	0.9812
77	0.2836	1.4566	1.3943
78	0.2642	1.1306	0.8133
79	0.1920	1.1136	1.1841
80	0.2721	1.2886	1.1893
81	0.2517	1.1000	0.7767
82	0.3363	0.7698	0.9942
83	0.3490	1.4401	1.1775
84	0.3483	0.9889	1.1399
85	0.0724	0.8714	1.0437
86	0.3253	0.8598	0.4137
87	0.1818	0.9091	0.9762
88	0.2174	0.7470	1.3040
89	0.2515	1.5077	1.1558
90	0.2880	1.3435	1.5973
91	0.2513	1.3623	1.3618
92	0.2132	0.7785	1.1535
93	0.2083	1.6554	0.5037
94	0.1910	0.8438	0.9521
95	0.2691	1.1157	1.1289
96	0.1150	0.7225	1.3708
97	0.1421	0.9262	0.7518
98	0.2487	0.4657	0.9828
99	0.0541	0.8815	0.9048
100	0.1523	0.7625	0.2739
Result			
Minimum	**0.0484 g**	**0.4657 g**	**0.2739 g**
Maximum	**0.5256 g**	**1.8614 g**	**2.6697 g**
Average	**0.2870 g**	**1.1636 g**	**1.4718 g**

4.8 Specific Gravity of *Mucuna* sps

The specific gravity of *Mucuna* sps extract with different solvents is depicted in Table No. 4.9. These results show that all the species almost same specific gravity in each solvent.

Table 4.9: Specific gravity of *Mucuna* sps extract with different solvents

Sr. No.	Solvents	Specific gravity of seeds		
		***Mucuna pruriens* (L.) DC**	***Mucuna cochinchinensis* (Lour.) A. Chev. (white Seed)**	***Mucuna cochinchinensis* (Lour.) A. Chev. (Black Seed)**
1	Water	0.9831	0.9698	0.9739
2	Methanol	0.7801	0.5151	0.7669
3	Ethanol	0.7773	0.8160	0..7655
4	Acetone	0.7719	0.7935	0.7586
5	Ethyl acetate	0.8851	0.9001	0.8700
6	Petroleum ether	0.6518	0.6607	0.6452
7	Benzene	0.8570	0.8738	0.8574
8	Chloroform	1.4539	1.4454	1.4266
9	Carbon tetra chloride	1.5526	1.5521	1.5176

4.9 Qualitative analysis:

In qualitative analysis of *Mucuna* sps it was found that, methanolic and water extracts showed the presence of alkaloids (*Wagner's test*) and phenols. When protein presence was checked using *lead acetate test* only methanolic extract gave positive detection. Hexose sugar was found only in methanolic extract of *M. pruriens.* Carbohydrates, reducing sugar, saponins, flavonoids (*lead acetate test*), alkaloids (*Mayer's test*) and starch was detected when water extract of the samples were analyzed. Volatile oil, organic acids, mucilage glycosides, pentose sugar and Cysteine amino acid was not detected in any of the sample and extract (Table 4.10).

Table 4.10: Qualitative analysis of *Mucuna* sps

Sr. No.	TEST	Different test Method	*M. pruriens*		*M. cochinchinensis* (White seed)		*M. cochinchinensis* (Black seed)	
			Methanolic Extract	Water Extract	Methanolic Extract	Water Extract	Methanolic Extract	Water Extract
1)	Test for Carbohydrates	Molish's test	-	+ve	-	+ve	-	+ve
2)	Test for reducing sugar	Fehling's test	-ve	+ve	-ve	+ve	-ve	+ve
3)	Test for pentose sugar	a)Phloroglucinol reag. Test	-ve	-ve	-ve	-ve	-ve	-ve
4)	Test for hexose sugar	a)Tollen's Phloroglucinol test	+ve	-ve	-ve	-ve	-ve	-ve
5)	Test for Protein	a) Biuret test	-ve	-ve	-ve	-ve	-ve	-ve
		b)Millon's test	-ve	-ve	-ve	-ve	-ve	-ve
		c)Xanthoprotein test	-ve	-ve	-ve	-ve	-ve	-ve
		d)Heat test	-ve	-ve	-ve	-ve	-ve	-ve
		5% Lead acetate	+ve	-ve	+ve	-ve	+ve	-ve
		5% Ammonium sulphate	-ve	+ve	-ve	-ve	-ve	-ve
6)	Test for Amino acid	a)Ninhydrin test	+ve	+ve	+ve	+ve	+ve	+ve
		b)Test for cysteine	-ve	-ve	-ve	-ve	-ve	-ve
7)	Test for Steroids	a)Libermann-Burchard Test	+ve	+ve	-ve	-ve	-ve	-ve
		b)Salkowski reaction	+ve	-ve	-ve	-ve	-ve	-ve
8)	Test for Glycoside	General test	-	-	-	-	-	-
9)	Test for Cardiac Glycosides	a)Legal test	-ve	-ve	-ve	-ve	-ve	-ve
		b)Keller-Killiani test	-ve	-ve	-ve	-ve	-ve	-ve

10)	Test for Anthroquinone glycoside	a)Borntrager's test	-ve	-ve	-ve	-ve	-ve	-ve
11)	Test for Cyanogenetic glycoside.	a)sodium picrate test	-	-	-	-	-	-
		b)mercurous nitate test	-	-	-	-	-	-
12)	Test for Coumarin glycoside	a)No aromatic odor	-ve	-ve	-ve	-ve	-ve	-ve
		b)No green fluorescence	-ve	-ve	-ve	-ve	-ve	-ve
13)	Test for Saponins	a)Foam test	-ve	+ve	-	+ve	-	+ve
		b)Lead acetate test	-ve	-ve	-ve	-ve	-ve	-ve
14)	Test for Flavonoids	a)Shinoda test	-ve	-ve	-ve	-ve	-ve	-ve
		b)Lead acetate test	-ve	+ve	+ve	-	+ve	-
		c)Alkaline reagent test	-ve	-ve	-ve	-ve	-ve	-ve
15)	Test for Alkaloids	a) Dragendorff's test	-ve	-ve	-ve	-ve	-ve	-ve
		b)Mayer's test	-ve	+ve	+ve	-ve	+ve	-ve
		c)Wagner's test	+ve	+ve	+ve	+ve	+ve	+ve
16)	Teat for Tannins	Lead acetate test	-ve	-ve	+ve	-ve	+ve	-ve
17)	Test for Mucilage		-ve	-ve	-ve	-ve	-ve	-ve
18)	Test for Phenol	a)$Fecl_3$ test	+ve	+ve	+ve	+ve	+ve	+ve
		b)Folin-ciocaltaue reagent	+ve	+ve	+ve	+ve	+ve	+ve
19)	Test for Starch	Iodine test	-	+ve	-	+ve	-	+ve
20)	Test for Organic acids	Calcium Chloride test	-ve	-ve	-ve	-ve	-ve	-ve
21)	Test for Volatile Oil		-ve	-	-ve		-ve	

4.10 Quantitative analysis of *Mucuna* sps.

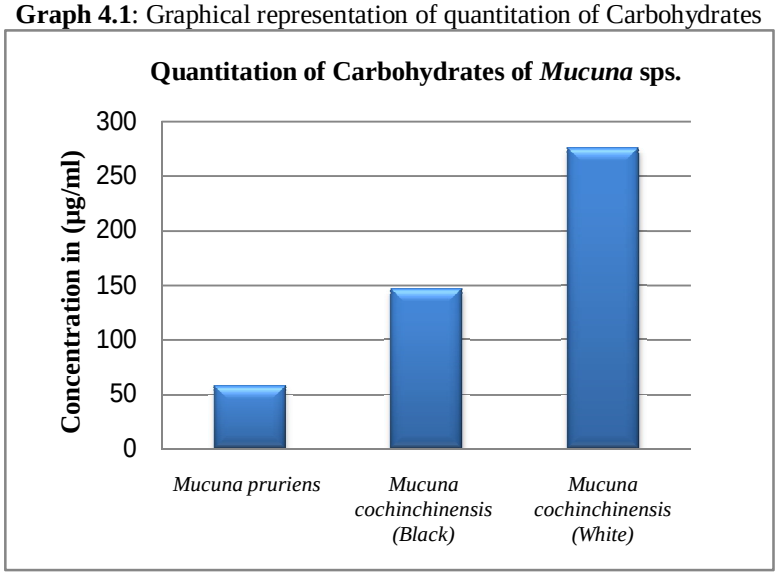
The screening and quantitative analysis using water extract of *Mucuna* sps for the carbohydrates, flavonoids, tannins, lipids, proteins, total phenolic content and glycosides is presented and expressed in Table 4.11 followed by respective graphs (Graphs 1-7).

The results of the quantitative phytochemical analysis showed that the seeds *M. cochienchinensis* white seed variety are rich in carbohydrates, flavonoids, and tannins whereas *M. cochienchinensis* black seed variety showed significant quantity of proteins and total phenolic contents. In the quantification of lipid content and glycosides (though the amount was significantly low in all the species under study) were present in maximum amount in *M. pruriens*.

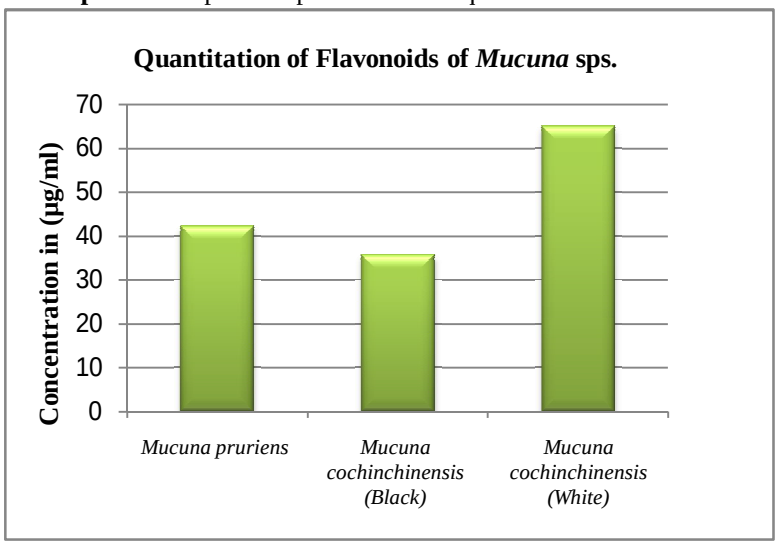
Table 4.11: Concentrations of various phyto-constituents in *Mucuna* sps

Sr. No.	Phyto-constituents	*M. pruriens* (µg/ml)	*M. cochinchinensis* (White Seed) (µg/ml)	*M. cochinchinensis* (Black Seed) (µg/ml)
1	Carbohydrates	57.4	146.6	275.8
2	Flavonoids	42.18	35.6	65.0
3	Tannins	203.6	306.2	576.7
4	Glycosides	56.28	10.16	32.8
5	Proteins	110.41	314	270.27
6	Total Phenolic content	10.42	80.68	40.00
7	Lipids	39.2	9.12	10.41

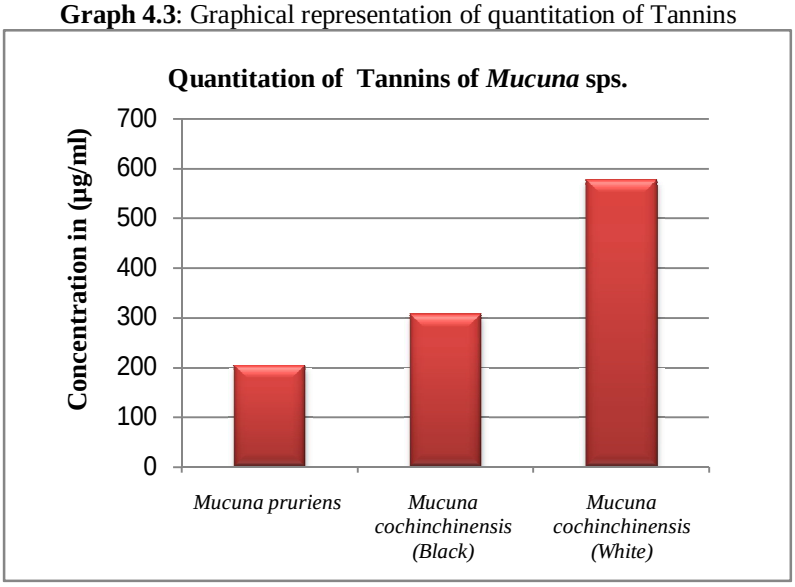
Graph 4.1: Graphical representation of quantitation of Carbohydrates



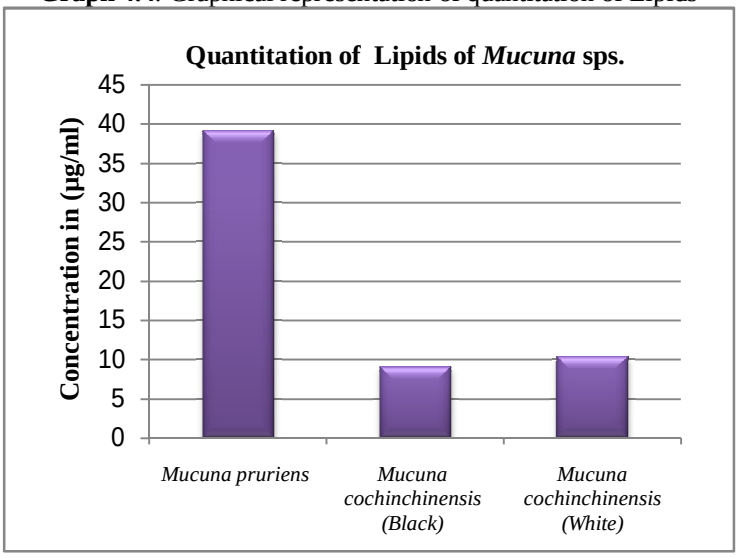
Graph 4.2: Graphical representation of quantitation of Flavonoids



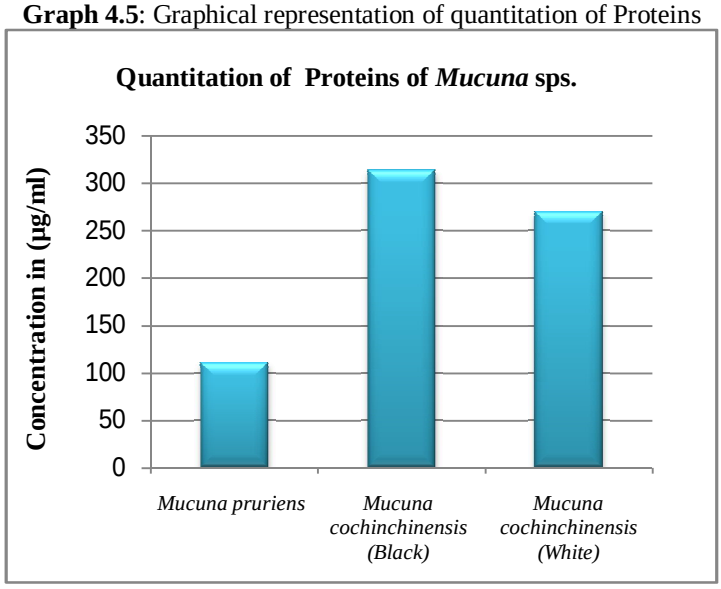
Graph 4.3: Graphical representation of quantitation of Tannins



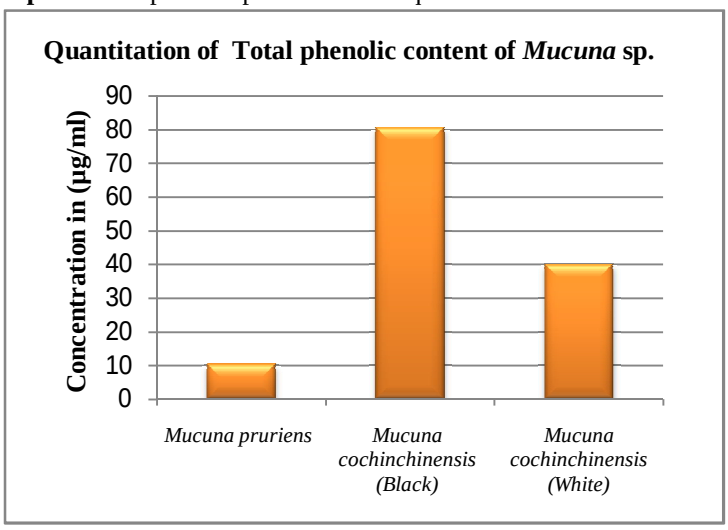
Graph 4.4: Graphical representation of quantitation of Lipids



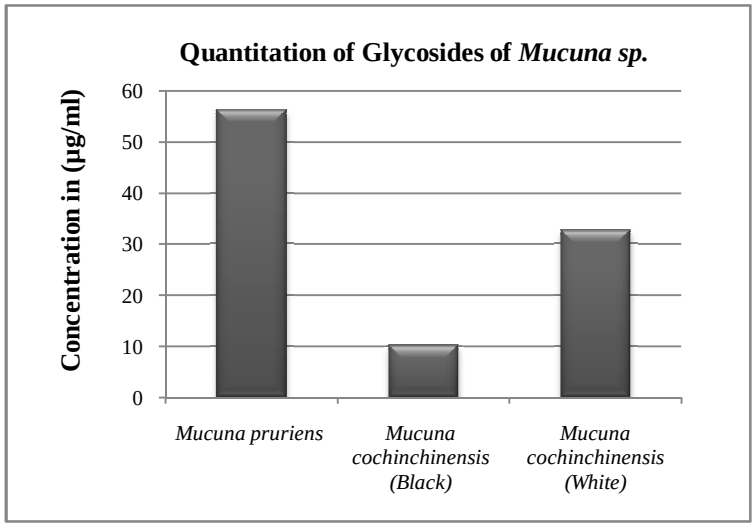
Graph 4.5: Graphical representation of quantitation of Proteins



Graph 4.6: Graphical representation of quantitation of Phenolic content



Graph 4.7: Graphical representation of quantitation of Glycosides



4.11 Elemental Analysis:

Table 4.12: Elemental analysis of *Mucuna* sps.

Sr. No.	Parameters	*Mucuna pruriens* (L.) DC.	*Mucuna cochinchinensis* (Lour.) A. Chev. (White Seed)	*Mucuna cochinchinensis* (Lour.) A. Chev. (Black Seed)
1	Nitrogen	5.22%	4.25%	4.61%
2	Phosphorus	0.35%	0.32%	0.32%
3	Potassium	1.15%	1.20%	1.10%
4	Calcium	0.024%	0.016%	0.020%
5	Magnesium	0.19%	0.16%	0.18%
6	Sulphur	0.29%	0.26%	0.21%
7	Iron	19ppm	18 ppm	15ppm
8	Manganese	29ppm	5 ppm	16ppm
9	Zinc	40ppm	34 ppm	32ppm
10	Copper	18ppm	19 ppm	15ppm
11	Sodium	0.25%	0.20%	0.20%

4.12 Thin Layer Chromatography (TLC)

A) *Mucuna pruriens* (L.) DC.

1. Methanolic extract

Using HPTLC applicator apply 10 µl of test solution as a bands on a precoated silica gel 60 F_{254} TLC plate (E. Merck) of uniform thickness of 0.2 mm and a height of 10 mm from the base of a 10×5 cm TLC. Develop the plate in the solvent system in a twin trough chamber to a distance of 8 cm from the base of the plate using the mobile phase-Pet-ether: Ethyl acetate (9:1). Observed the plate under UV-254 nm shows 02 bands, Rf value and color of the band is at 0.10 (Black),0.19 (Black);Under UV-366 nm band is not observed and after spraying the plate with Anisaldehyde-sulphuric acid reagent followed by heating at 105^0C for 10 minutes, shows 10 bands, Rf value and color of the bands are at 0.13 (Purple),0.19(Yellow),0.23 (Blue),0.28 (Pink),0.36 (Purple), 0.47 (Pink), 0.58 (Purple),0.68 (Pink),0.76 (Pink),0.87 (Pink).

2. Petroleum ether extract-

Using HPTLC applicator apply 10 µl of test solution as a bands on a precoated silica gel 60 F_{254} TLC plate (E. Merck) of uniform thickness of 0.2 mm and a height of 10 mm from the base of a 10×5 cm TLC. Develop the plate in the solvent system in a twin trough chamber to a distance of 8 cm from the base of the plate using the mobile phase-Pet-ether: Ethyl acetate (9:1). Observed the plate under UV-254 nm

shows 02 bands, Rf value and color of the band is at 0.44 (Black),0.88(Black);Under UV-366 nm band is not observed and after spraying the plate with Anisaldehyde-sulphuric acid reagent followed by heating at 105^0C for 10 minutes, shows 07 bands, Rf value and color of the bands are at 0.13 (Pink), 0.19 (Pink),0.26 (Gray),0.44 (Pink),0.54 (Pink), 0.68 (Pink), 0.88 (Pink).

B) *Mucuna cochinchinensis* (Lour.) A. Chev (Black seeds):

1) Methanolic extract

Using HPTLC applicator apply 10 µl of test solution as a bands on a precoated silica gel 60 F_{254} TLC plate (E. Merck) of uniform thickness of 0.2 mm and a height of 10 mm from the base of a 10×5 cm TLC. Develop the plate in the solvent system in a twin trough chamber to a distance of 8 cm from the base of the plate using the mobile phase-Toluene: Ethyl acetate: Methanol (8:1.5:0.5). Observed the plate under UV-254 nm shows 01 band, Rf value and colour of the band is at 0.18 (Black); Under UV-366 nm band is not observed and after spraying the plate with Anisaldehyde-sulphuric acid reagent followed by heating at 105^0C for 10 minutes, shows 10 bands, Rf value and colour of the bands are at 0.08 (Pink), 0.14(Purple), 0.20 (Brown), 0.28 (Purple), 0.31(Pink), 0.54 (Pink), 0.63 (Gray), 0.74 (Pink),0.83(Pink),0.94 (Pink).

2) Petroleum ether extract

Using HPTLC applicator apply 10 µl of test solution as a bands on a precoated silica gel 60 F_{254} TLC plate (E. Merck) of uniform thickness of 0.2 mm and a height of 10 mm from the base of a 10×5 cm TLC. Develop the plate in the solvent system in a twin trough chamber to a distance of 8 cm from the base of the plate using the mobile phase-Pet-ether: Ethyl acetate (9:1). Observed the plate under UV-254 nm shows 02 bands, Rf value and color of the band is at 0.41 (Black),0.63 (Black);Under UV-366 nm band is not observed and after spraying the plate with Anisaldehyde-sulphuric acid reagent followed by heating at 105^0C for 10 minutes, shows 11bands, Rf value and color of the bands are at 0.06 (Brown),0.09 (Pink),0.13 (Pink),0.17 (Pink),0.21 (Brown), 0.27 (Pink), 0.33 (Pink),0.41 (Purple),0.49 (Pink),0.63 (Pink).0.91 (Pink).

C) *Mucuna cochinchinensis* (Lour.) A. Chev. (White Seed)

1. Methonolic extract

Using HPTLC applicator apply 10 µl of test solution as a bands on a precoated silica gel 60 F_{254} TLC plate (E. Merck) of uniform thickness of 0.2 mm and a height of 10 mm from the base of a 10×5 cm TLC. Develop the plate in the solvent system in a twin trough chamber to a distance of 8 cm from the base of the plate using the mobile phase-Toluene: Ethyl acetate: Methanol (8:1.5:0.5).Observed the plate under UV-254 nm show 02 band, R_f value and color of the band is at 0.10 (Black) and 0.19 (Black), Under UV-366 nm band is not observed and after spraying the plate with Anisaldehyde-sulphuric acid reagent followed by heating at 105^0C for 10 minutes, shows 10 bands, R_f value and color of the bands are at 0.10 (Blue),0.19 (Purple), 0.23 (Pink),0.49 (Pink),0.60 (Blue),0.77 (Pink), 0.80 (Pink),0.87 (Pink), 0.90(Brown),0.92 (Pink).

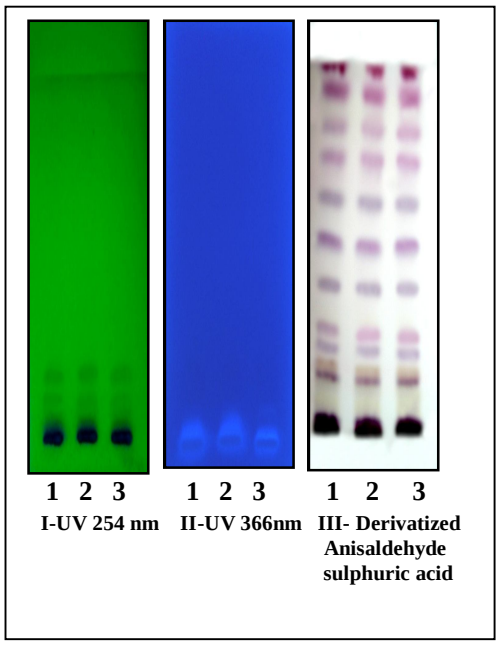
2. Petroleum ether extract

Using HPTLC applicator apply 10 µl of test solution as a bands on a precoated silica gel 60 F_{254} TLC plate (E. Merck) of uniform thickness of 0.2 mm and a height of 10 mm from the base of a 10×5 cm TLC. Develop the plate in the solvent system in a twin trough chamber to a distance of 8 cm from the base of the plate using the mobile phase- Pet-ether: Ethyl acetate (9:1). Observed the plate under UV-254 nm shows 02 bands, Rf value and color of the band is at 0.49 (Black),0.53(Black); Under UV-366 nm band is not observed and after spraying the plate with Anisaldehyde-sulphuric acid reagent followed by heating at 105^0C for 10 minutes, shows 12 bands, Rf value and color of the band are at 0.01 (Green), 0.03 (Purple), 0.13 (Green), 0.23 (Gray),0.34 (Green),0.38 (Green), 0.49 (Green), 0.53 (Purple), 0.60 (Green), 0.68 (Green), 0.79 (Green), 0.88 (Green).

Plate 24: TLC Profile of *Mucuna pruriens* (L.) DC.

TLC Profile of Methanolic extracts of seeds of *M. pruriens*

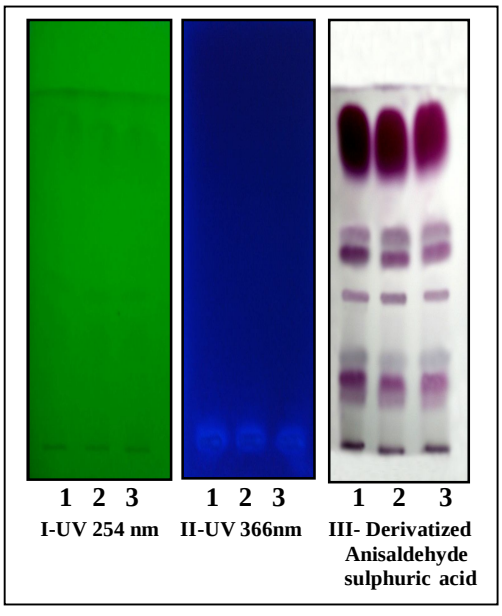
Mobile phase: Petroleum-ether: Ethyl acetate (9:1)



Sr. No.	I-UV-254nm		II-UV-366nm		III-After Derivatization (Anisaldehyde - sulphuric acid)	
	Rf Value	Colour of the band	Rf value	Colour of the band	Rf value	Colour of the band
1	0.10	Black			0.13	Purple
2	0.19	Black	-	-	0.19	Yellow
3					0.23	Blue
4					0.28	Pink
5					0.36	Purple
6					0.47	Pink
7					0.58	Purple
8					0.68	Pink
9					0.76	Pink
10					0.87	Pink

TLC Profile of Pet ether extracts of seeds of *Mucuna pruriens* (L.) DC.

Mobile phase: Petroleum-ether: Ethyl acetate (9:1)



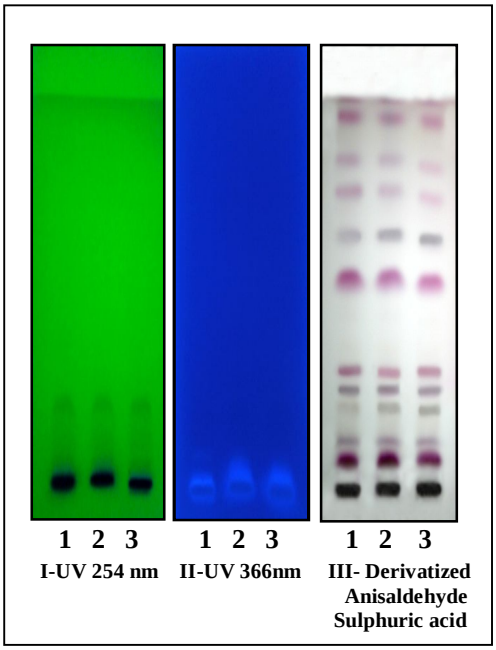
Sr. No.	I-UV-254nm		II-UV-366nm		III-After Derivatization (Anisaldehyde sulphuric acid)	
	Rf Value	Colour of the band	Rf value	Colour of the band	Rf value	Colour of the band
1	0.44	Black			0.13	Pink
2	0.88	Black	-	-	0.19	Pink
3					0.26	Gray
4					0.44	Pink
5					0.54	Pink
6					0.68	Pink
7					0.88	Pink

Plate 25:TLC Profile of *M. cochinchinensis* (Black seed)

TLC Profile of Methanolic extract of *M. cochinchinensis* (Black seed)

Mobile phase:

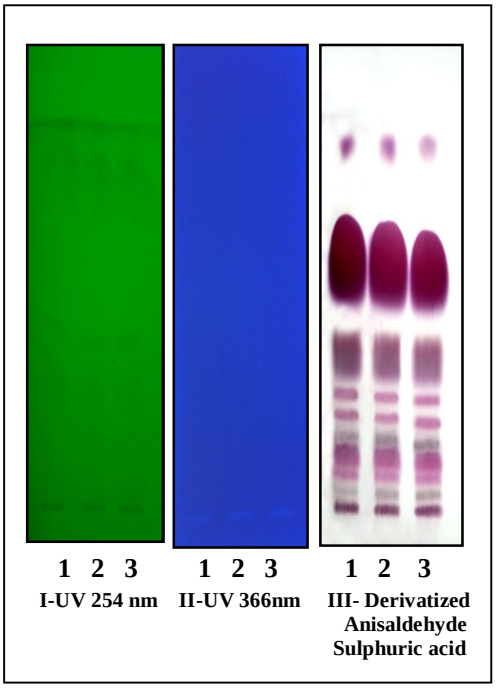
Toluene: Ethyl acetate: Methanol (8:1.5:0.5)



Sr. No.	I-UV-254nm		II-UV-366nm		III-After Derivatization (Anisaldehyde-sulphuric acid)	
	Rf Value	Colour of the band	Rf value	Colour of the band	Rf value	Colour of the band
1	0.18	Black	-	-	0.08	Pink
2					0.14	Purple
3					0.20	Brown
4					0.28	Purple
5					0.31	Pink
6					0.54	Pink
7					0.63	Gray
8					0.74	Pink
9					0.83	Pink
10					0.94	Pink

TLC Profile of Pet ether extracts of Black Seeds of *M. cochinchinensis*

Mobile phase: Petroleum-ether: Ethyl acetate (9:1)



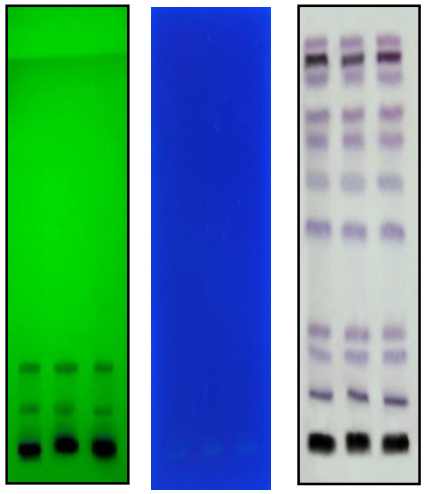
Sr. No.	I-UV-254nm		II-UV-366nm		III-After Derivatization (Anisaldehyde sulphuric acid)	
	Rf Value	Colour of the band	Rf value	Colour of the band	Rf value	Colour of the band
1	0.41	Black			0.06	Brown
2	0.63	Black	-	-	0.09	Pink
3					0.13	Pink
4					0.17	Pink
5					0.21	Brown
6					0.27	Pink
7					0.33	Pink
8					0.41	Purple
9					0.49	Pink
10					0.63	Pink
11					0.91	Pink

Plate 26: TLC Profile of *Mucuna cochinchinensis* (White seeds)

TLC Profile of Methanolic extract of White Seeds of *M. cochinchinensis*

Mobile phase:

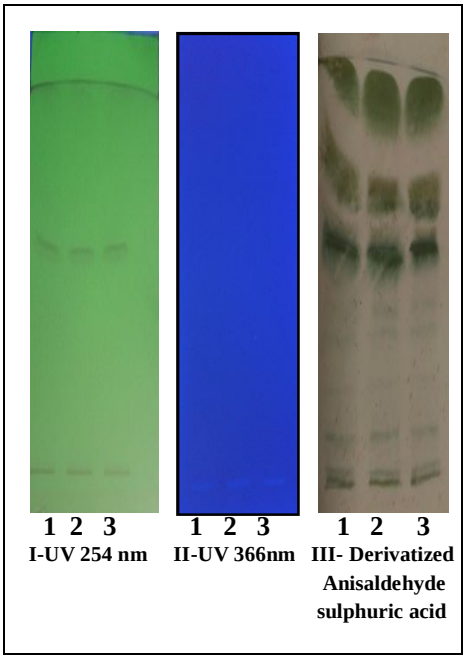
Toluene: Ethyl acetate: Methanol (8:1.5:0.5)



S.N.	I-UV-254nm		II-UV-366nm		III-After Derivatization (Anisaldehyde - sulphuric acid)	
	R_f Value	Colour of the Band	R_f value	Colour of the band	R_f value	Colour of the band
1	0.10	Black	-	-	0.10	Blue
2	0.19	Black			0.19	Purple
3					0.23	Pink
4					0.49	Pink
5					0.60	Blue
6					0.77	Pink
7					0.80	Pink
8					0.87	Pink
9					0.90	Brown
10					0.92	Pink

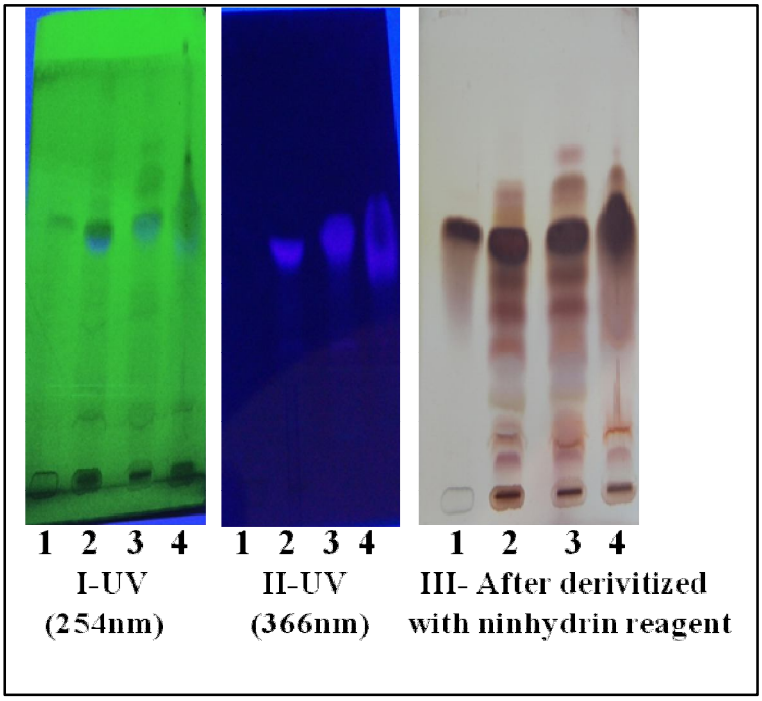
TLC Profile of Pet ether extract of White Seeds of *Mucuna cochinchinensis* (Lour.) A. Chev.

Mobile phase: Petroleum-ether: Ethyl acetate (9:1)



Sr. No.	I-UV-254nm		II-UV-366nm		III-After Derivatization (Anisaldehyde - sulphuric acid)	
	R_f Value	Colour of the Band	R_f value	Colour of the band	R_f value	Colour of the band
1	0.49	Black	-	-	0.01	Green
2	0.53	Black	-	-	0.03	Purple
3					0.13	Green
4					0.23	Grey
5					0.34	Green
6					0.38	Green
7					0.49	Green
8					0.53	Purple
9					0.60	Green
10					0.68	Green
11					0.79	Green
12					0.88	Green

Plate 27: TLC Profile of aqueous seed extracts of *Mucuna* sps. with Standard L-Dopa



Where,

1 Standard (L-dopa)
2 *M. Pruriens* (L.) DC.
3 *M. Cochinchinensis* (Lour.) A. Chev. (Black seed)
4 *M. Cochinchinensis* (Lour.) A. Chev. (White seed)

TLC detail of Aqueous extracts of seeds of *Mucuna sps* with L-Dopa

Sr. No.	*Mucuna pruriens* (L.) DC.						*Mucuna cochinchinensis* (Lour.) A. Chev. (Black Seed)						*Mucuna cochinchinensis* (Lour.) A. Chev. (White Seed)						Standard L-Dopa
	I-UV-254nm		II-UV-366nm		III-After Derivatization (ninhydrin reagent)		I-UV-254nm		II-UV-366nm		III-After Derivatization (ninhydrin reagent)		I-UV-254nm		II-UV-366nm		III-After Derivatization (ninhydrin reagent)		After Derivatization (ninhydrin reagent)
	R_f Value	Colour of the band	R_f value	Colour of the band	R_f value	Colour of the band	R_f Value	Colour of the band	R_f value	Colour of the band	R_f value	Colour of the band	R_f Value	Colour of the band	R_f value	Colour of the band	R_f value	Colour of the band	
1	0.11	Black	0.52	Fluroscent blue	0.07	Pink	0.11	Black	0.52	Fluroscent blue	0.08	Pink	0.11	Black	0.52	Fluroscent blue	0.08	Light Pink	0.52
2	0.52	Blue			0.11	Red	0.52	Blue			0.14	Red	0.52	Blue			0.12	Red	
3					0.17	Yellow					0.21	Light yellow					0.20	Light yellow	
4					0.20	Light yellow					0.24	Light Purple					0.32	Pink	
5					0.31	Pink					0.30	Pink					0.40	Light Purple	
6					0.39	Yellowish Brown					0.32	LightBrown					0.48	LightBrown	
7					0.44	LightBrown					0.44	Brown					0.52	Brown	
8					0.52	Brown					0.52	Brown					0.56	LightBrown	
9					0.58	Yellow					0.64	LightBrown					0.67	Brown	
10					0.63	Pink					0.70	Pink					0.72	Pink	

C. Quantification of L-Dopa in *Mucuna* sps

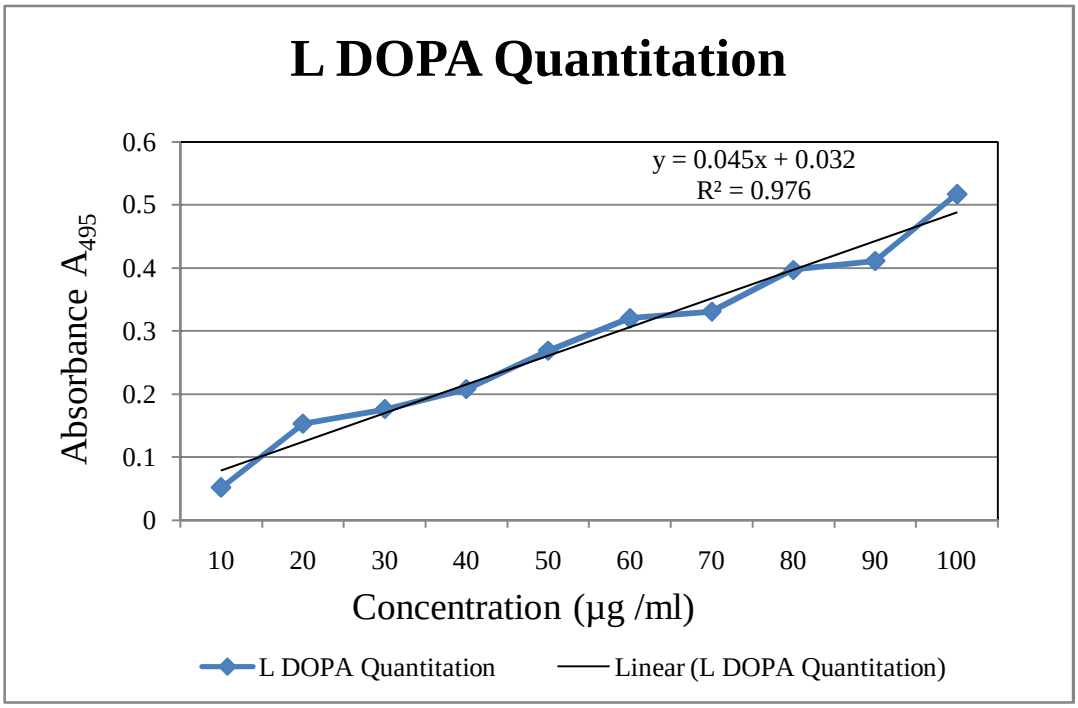
4.13 Quantification of L-Dopa

The quantification of L-Dopa in the aqueous seed extract of *Mucuna* sps was carried out by calorimetric method using ELISA reader. The observations are depicted in Table 4.13.

Table 4.13: L-dopa Quantitation of *Mucuna* (µg/ml)

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

Graph 4.8: Graphical representation of Standard plot of L – dopa obtained at Absorbance 476 nm.



D. DNA Fingerprinting by RAPD technique

4.14 RAPD-PCR analysis results

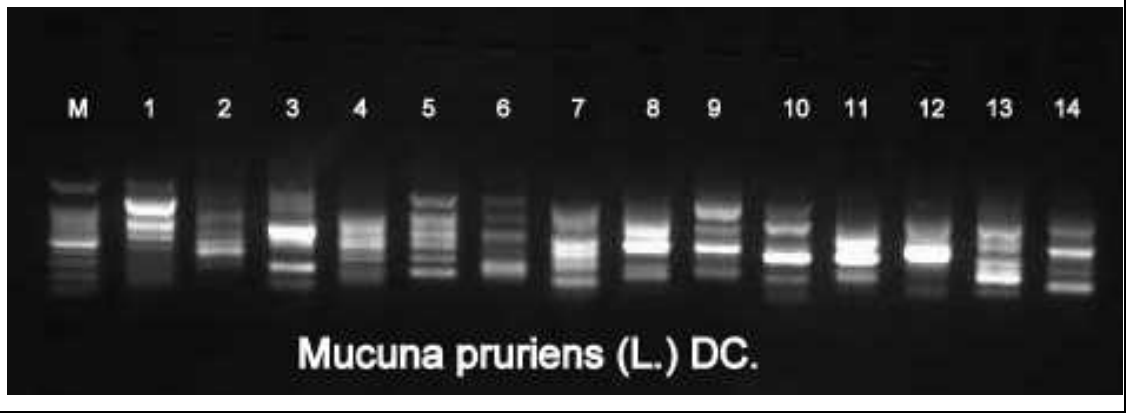
A successful DNA isolation was achieved for all *Mucuna* species under study. DNA extraction results are summarized in table 26.

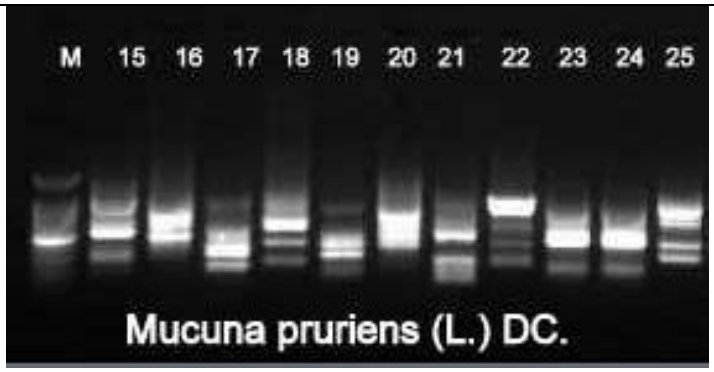
Table 4.14: Amount of DNA extracted

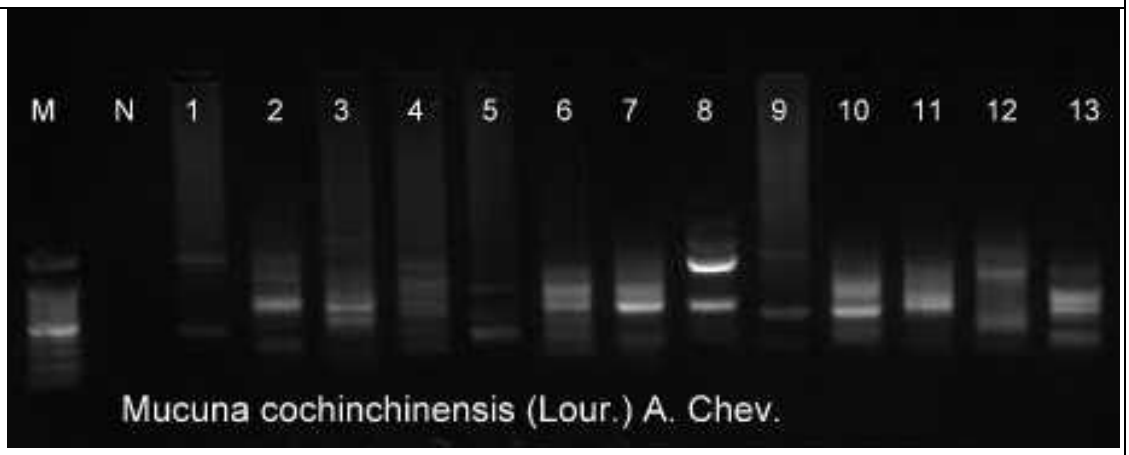
S. No.	Name of the species	Amount of DNA in ng/µl
1	*Mucuna pruriens*	21.32 ng/µl
2	*Mucuna cochinchinensis* (White seed)	20.5 ng/µl
3	*Mucuna cochinchinensis* (Black seed)	19.89 ng/µl

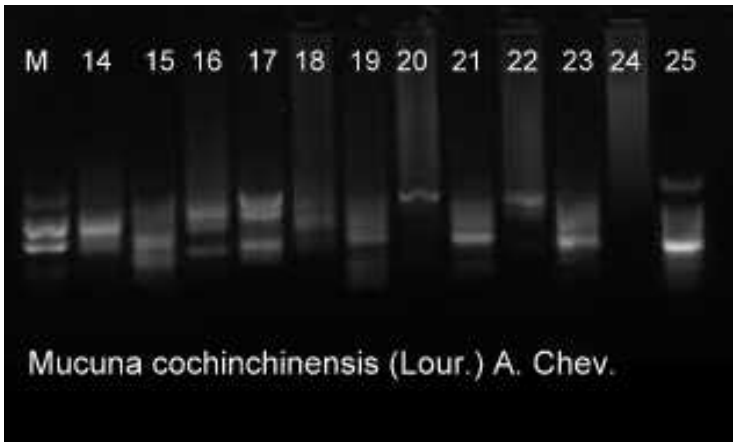
As evident from RAPD profiles, the two accessions selected of *Mucuna* could be distinguished at the molecular level. The comparative analysis of all accessions was established on the basis of RAPD profiles generated by using decanucleotide primers. Figure 4.1 shows the characteristic RAPD profile of *M. pruriens*, and also depicts the genetic differentiation of *M. cochinchinensis*. Only the primers which displayed prominent, reproducible and distinguishable bands were considered for analysis. The 14 tested primers yielded totally 107 scorable bands with an average of three bands per primer which showed polymorphism. 60% polymorphism was observed in *M. cochinchinensis* and 92% in *M. pruriens*. All primers showed amplification for *M. pruriens* whereas primer number 4, 18 and 24 did not show any bands in the RAPD profile of *M. cochinchinensis.*

Figure 4.1: RAPD profile of *Mucuna* sps




M 15 16 17 18 19 20 21 22 23 24 25
Mucuna pruriens (L.) DC.


M N 1 2 3 4 5 6 7 8 9 10 11 12 13
Mucuna cochinchinensis (Lour.) A. Chev.


M 14 15 16 17 18 19 20 21 22 23 24 25
Mucuna cochinchinensis (Lour.) A. Chev.

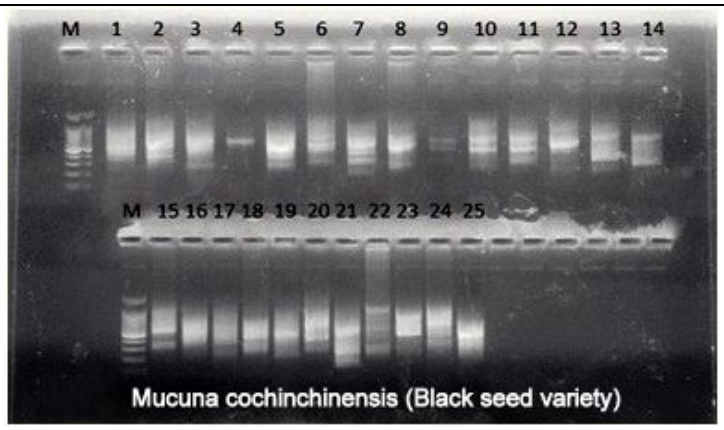
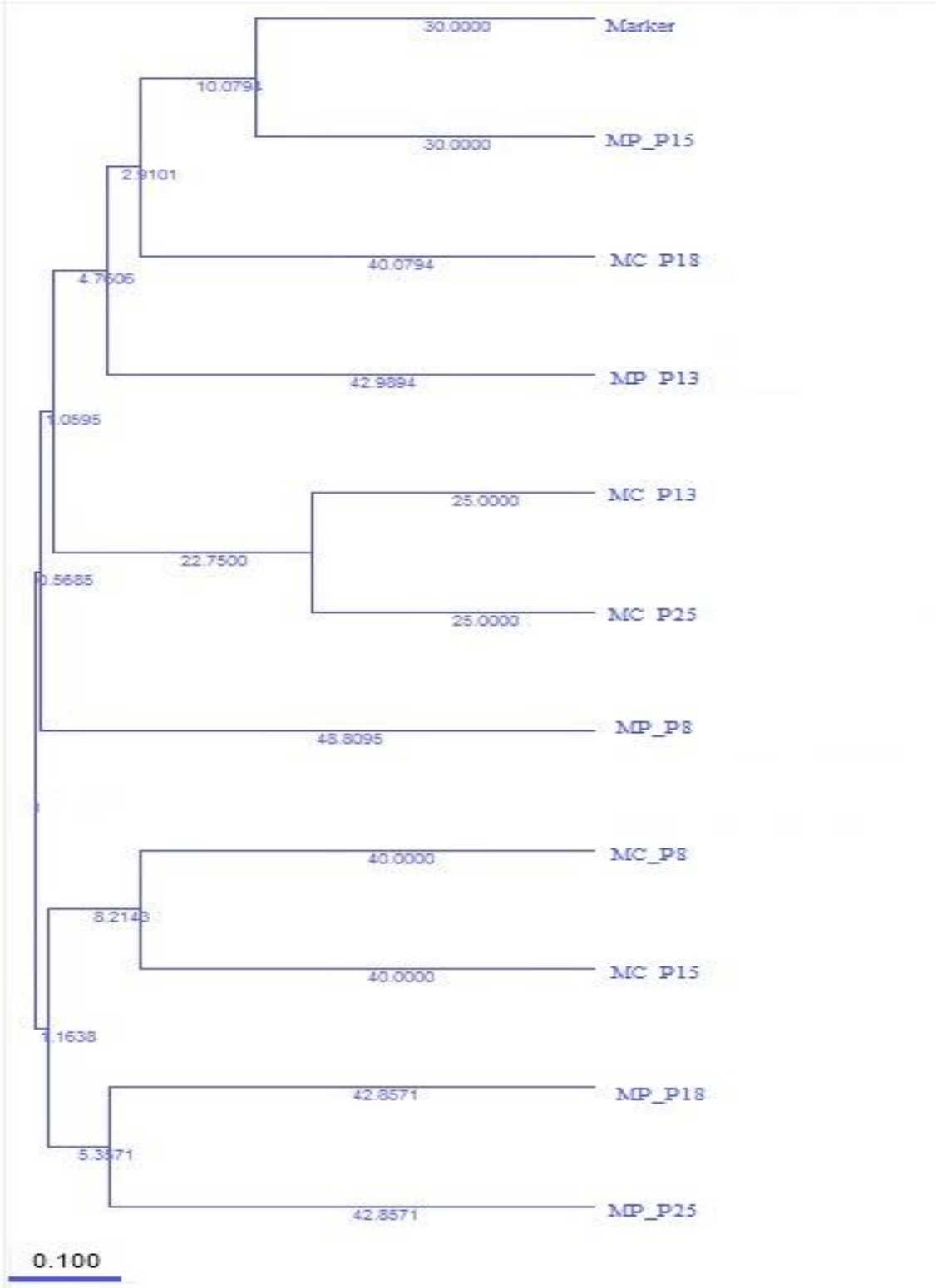

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14
M 15 16 17 18 19 20 21 22 23 24 25
Mucuna cochinchinensis (Black seed variety)

Figure 4.2: UPGMA phenogram



E. Pharmacological and Toxicological work

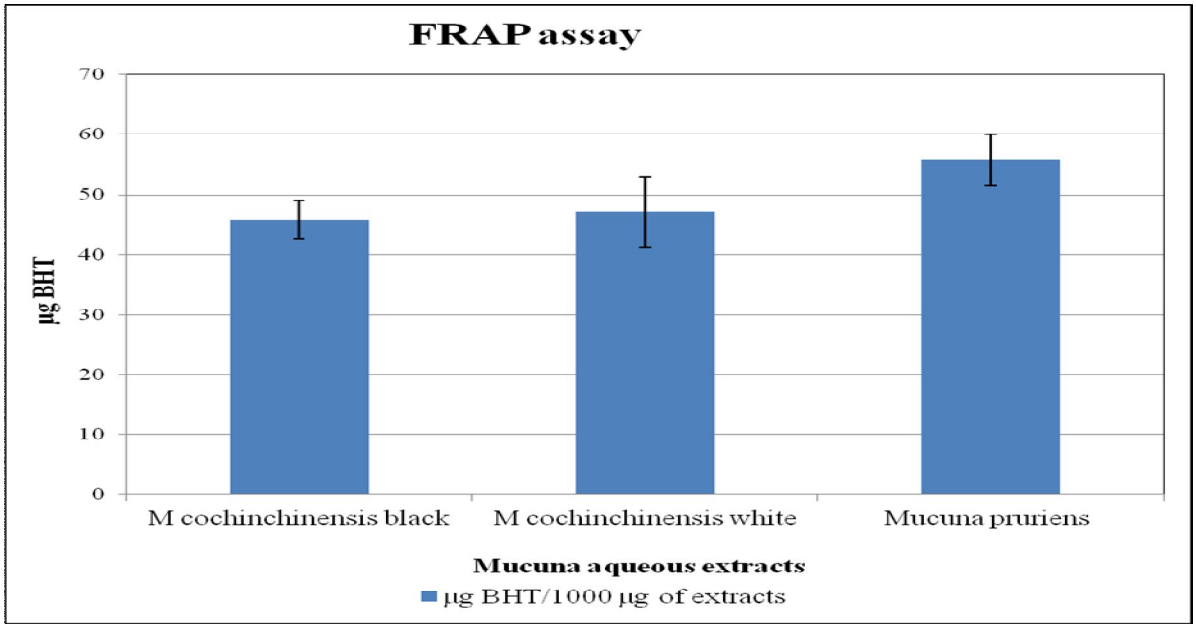
4.15 Antioxidant activity:

The essential chemical components of plant namely 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 4.9 and graph 9-12. *M. 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.

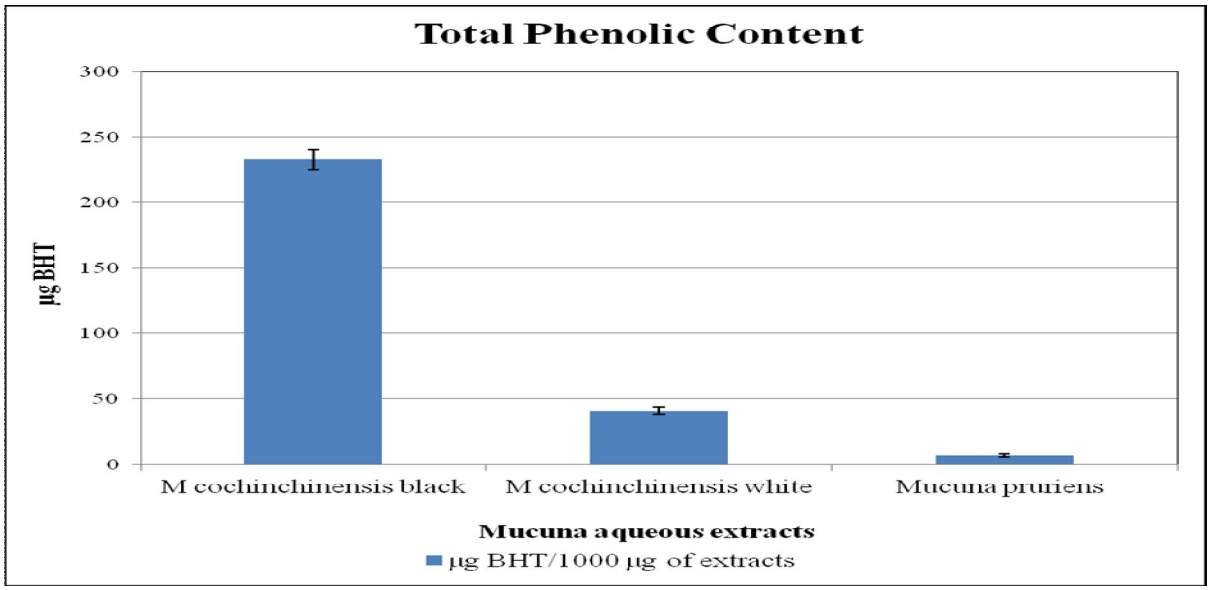
In DPPH radical scavenging assay extract of *M cochinchinensis* black seed had highest % inhibition activity at 1000µg/ml of extract (99.856%). In FRAP assay *M. pruriens* extract showed highest potential to reduce the ferric ions (equivalent of 55.74 µg BHT).

Table 4.15: Levels of Phytochemical and antioxidant potential of *Mucuna* sps

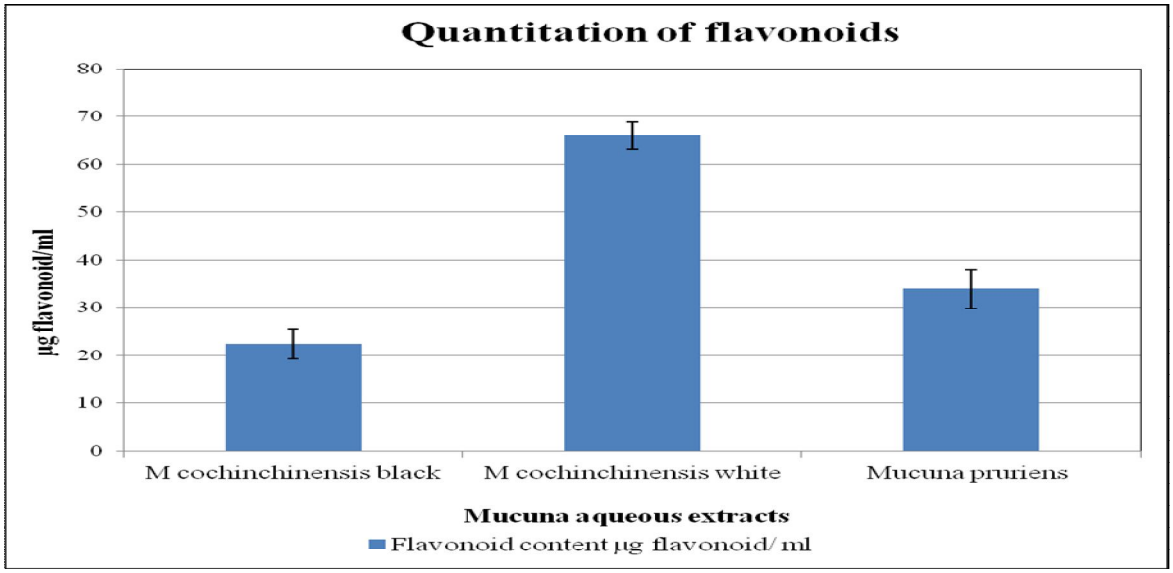
Sl. No.	Name of chemical test/assay	*M. cochinchinensis* Black seed	*M. cochinchinensis* White seed	*M. pruriens*
1	Total Phenolic Content (µg BHT/1000 µg of extracts)	233.085 ± 7.8219	41.06 ± 2.8151	7.336 ± 1.2312
2	Flavonoid assay (µg flavonoid/1000 µg of extracts)	22.43 ± 3.1259	66.13 ± 2.8152	33.84 ± 4.1266
3	DPPH assay (% Inhibition/1000 µg of extracts)	99.856 ± 5.1962	99.773 ± 6.9768	92.9135 ± 6.2851
4	FRAP antioxidant assay (µg BHT/1000 µg of extracts)	45.772 ± 3.2135	47.103 ± 5.9562	55.74 ± 4.2791



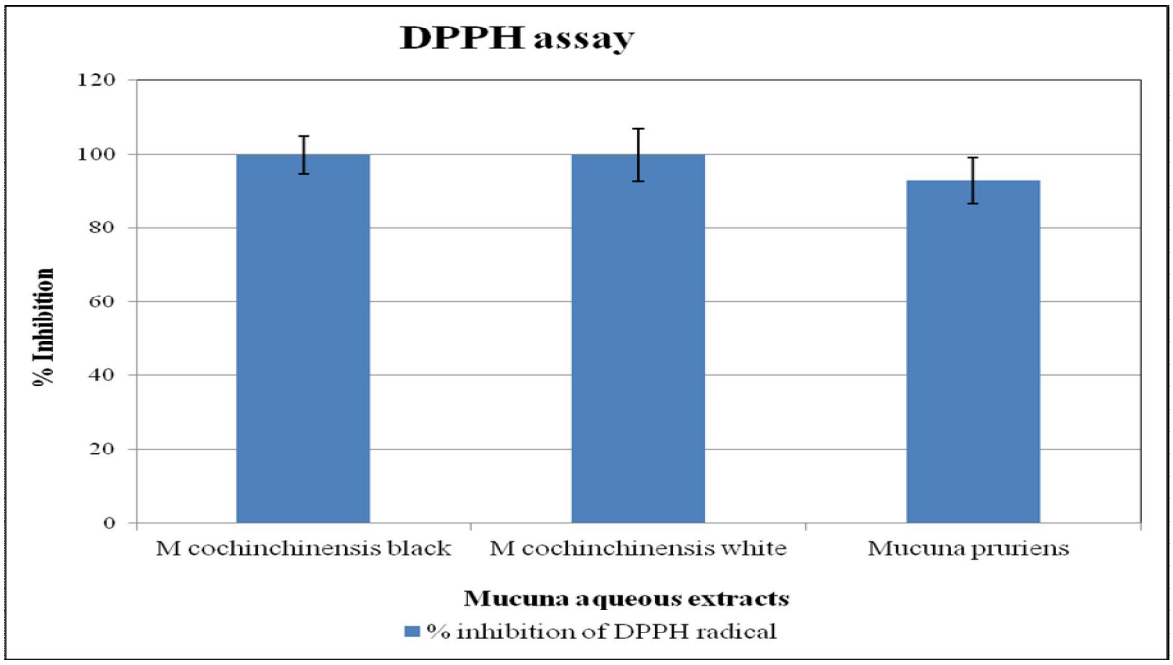
Graph 4.9: Antioxidant activity of *Mucuna* sps by FRAP assay



Graph 4.10: Total phenolic content of *Mucuna* sps



Graph 4.11: Flavonoid content of *Mucuna* sps



Graph 4.12: Antioxidant activity of *Mucuna* sps by DPPH radical inhibition assay

4.16 Spermatogenesis evaluation:

Survival and Clinical Signs

There was no incidence of treatment related mortality among the animals exposed to the standard drug and test materials (*Mucuna cochinchinensis* (Black seed), *Mucuna cochichinensis* (White seed) and *Mucuna pruriens*) at 100, 200, 400 mg/kg body weight. Treatment of rats with test materials at all doses did not induce any noticeable treatment related abnormal clinical signs.

Body Weights :

Mucuna cochichinensis (Black seed) and *Mucuna pruriens* at 400 mg/kg body weight dose and standard drug induce significant increase in the group mean body weights of the animals. Other doses at 100 and 200 mg/kg body weight of test drugs did not induce any observable change in body weight animals.

Food Consumption :

The values of average daily food consumption in all groups were comparable with control group of animals.

Necropsy :

All test materials (*Mucuna cochinchinensis* (Black seed), *Mucuna cochichinensis* (White seed) and *Mucuna pruriens*) at doses of 100, 200 and 400 mg/kg body weight and standard drug at 20 mg/kg dose did not induce any significant treatment related gross pathological changes in any of the organs or tissues of treated rats.

Organ Weights :

Increase in absolute and relative weights of testes and epididymis in group II, VIII and XI animals was found to be statistically significant ($p<0.05$) when compared with control group of animals.

Sperm count, motility and morphology of sperm :

Treatment with standard drug at 20 mg/kg and *Mucuna cochichinensis* (Black seed) and *Mucuna pruriens* at 400 mg/kg body weight for 30 days induces significant increase in sperm count, motility and morphology of sperms ($p< 0.05$).

Biochemical Analysis:

The rat serum levels of testosterone, luteinizing hormone and total antioxidant capacity showed significant increase after treatment with the aqueous extracts of *M.*

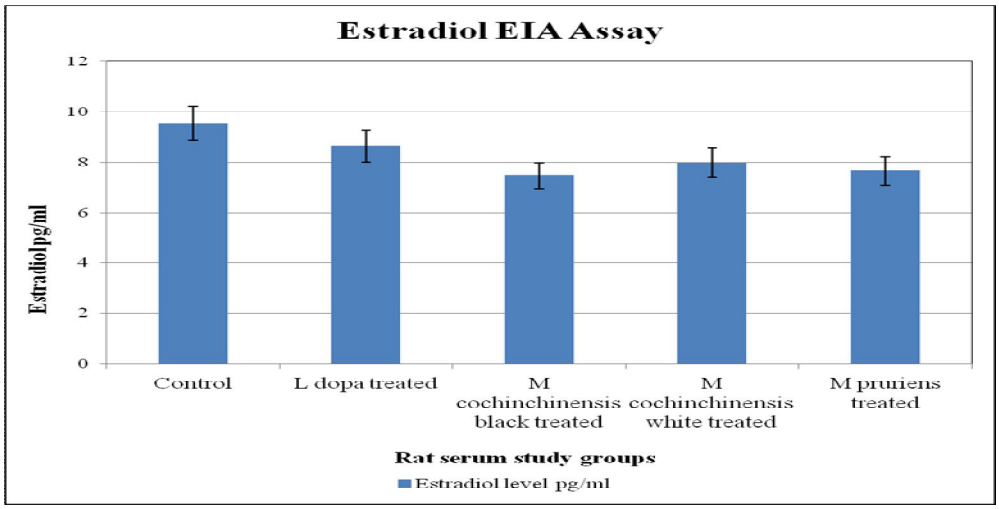
cochinchinensis (Black seed variety), *M. cochichinensis* (White seed variety) and *M. pruriens* at 400 mg/kg body weight for 30 days as compared to the normal control animals. The increase in serum levels of these biochemical parameters was comparable with those of L-Dopa treated animals. The level of rat serum estradiol in the treatment group was declined as compared to the normal control animals (Table 4.10 and Graph 13-16).

Histological section:

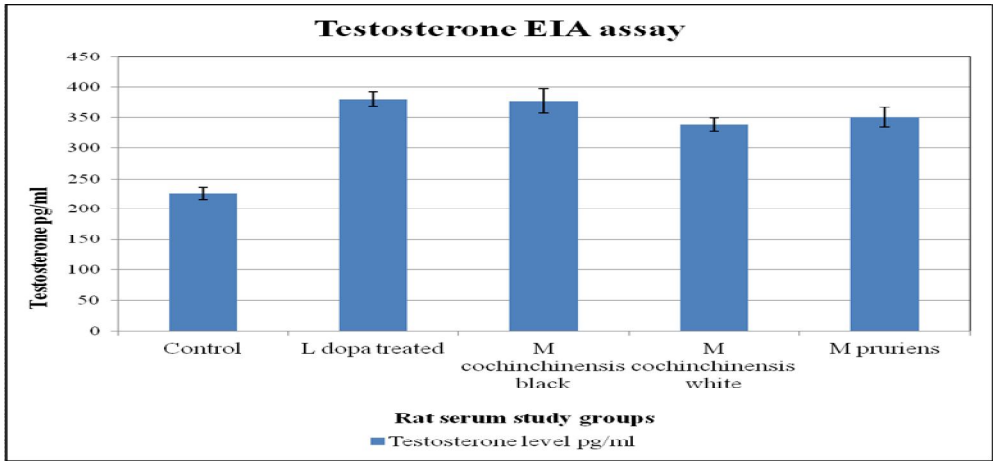
Cross section of testes in the aqueous extract treated groups with 400 mg/kg body weight of *M. cochinchinensis* (black seed variety) revealed more number of seminiferous tubules having maximum diameter, large number of different cells (sertoli, leydigs cells) at different stages of spermatogenesis and spermatozoa in seminiferous tubules (Fig 4.5). This profile was followed by sections of testes treated with *M. pruriens* and *M. cochinchinensis* white seed variety (Fig 4.8, 4.11). The histological cross sections of testes in the control group showed normal histological texture (Fig 4.13).

Table 4.16: Serum level of estradiol, testosterone, luteinizing hormone and total antioxidant capacity in treated and control rats serum

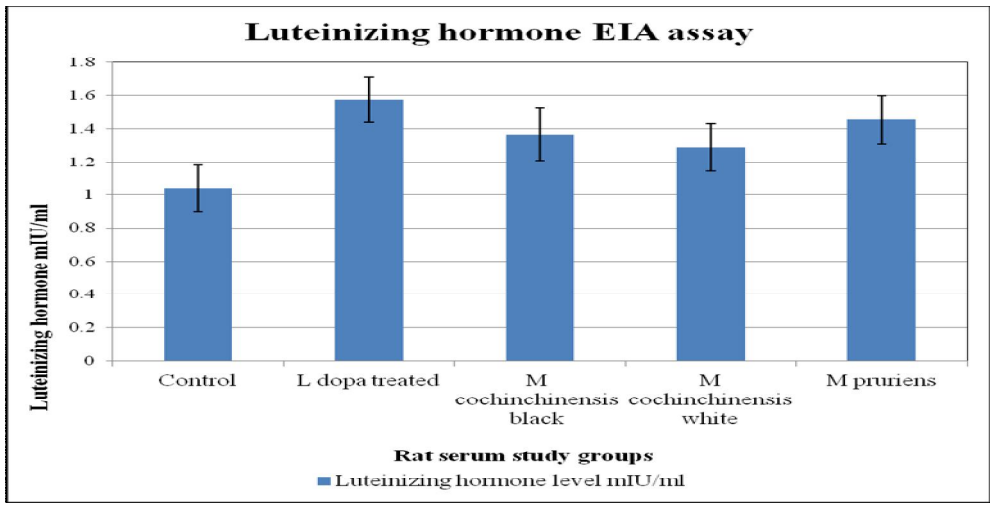
Sl. No.	Name of chemical test/assay	Control	L-Dopa	*M. cochinchinensis* (Black seed)	*M. cochinchinensis* (White seed)	*M. pruriens*
1	Estradiol EIA Assay	9.548±0.68	8.654±0.64	7.49±0.57	7.996±0.51	7.669±0.56
2	Testosterone EIA assay	225.42±10.35	380.55±11.55	377.14 ±19.55	338.15±10.92	350.84±16.55
3	Luteinizing hormone EIA assay	1.04±0.139	1.575±0.138	1.367±0.158	1.288±0.142	1.453±0.145
4	Total Antioxidant Capacity Assay	13.31±1.92	13.87±1.55	15.232±1.81	14.991±1.67	21.066±1.86



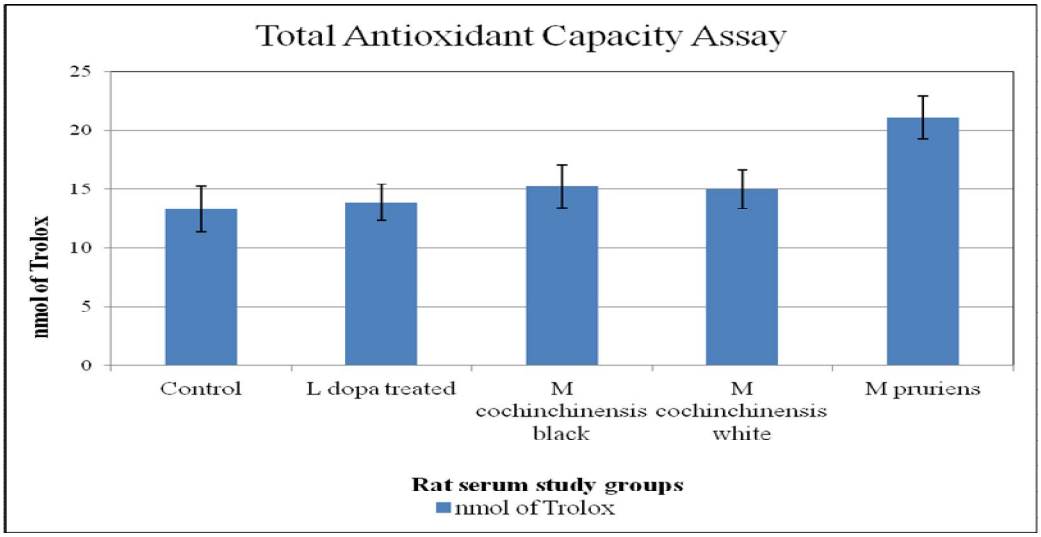
Graph 4.13: Serum Estradiol level in rats after treatment with aqueous extract of *Mucuna* sps.



Graph 4.14: Serum Testosterone level in rats after treatment with aqueous extract of *Mucuna* sps.



Graph 4.15: Serum LH level in rat after treatment with aqueous extract of *Mucuna* sps



Graph 4.16: Serum TAC level in rat after treatment with aqueous extract of *Mucuna* sps

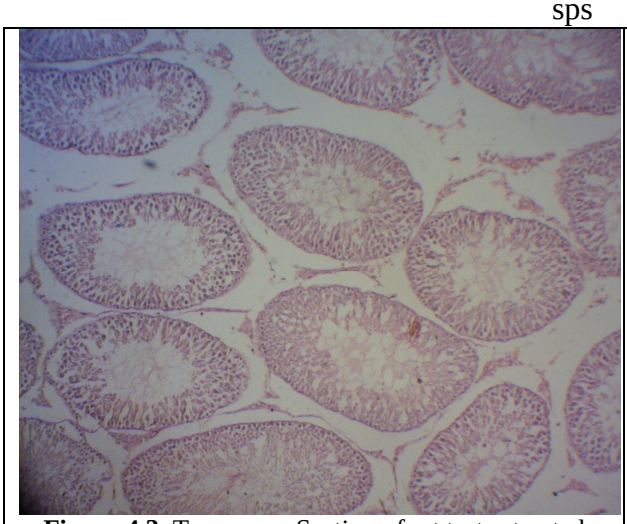


Figure 4.3. Transverse Section of rat testes treated with aqueous extract of *Mucuna cochinchinensis* (Black seed) at 100 mg/kg body weight, orally.

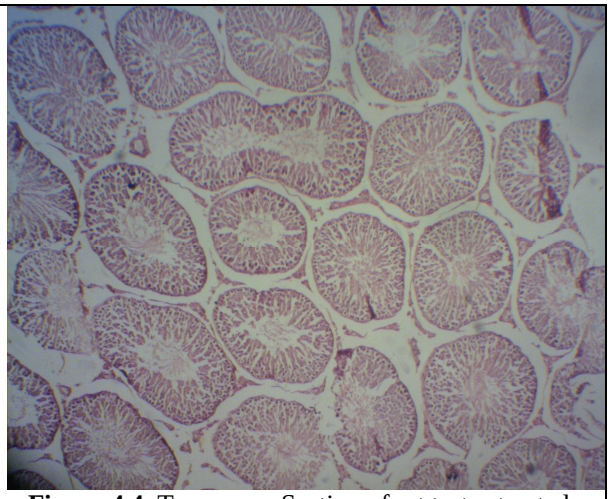


Figure 4.4. Transverse Section of rat testes treated with aqueous extract of *Mucuna cochinchinensis* (Black seed) at 200mg/kg body weight, orally.

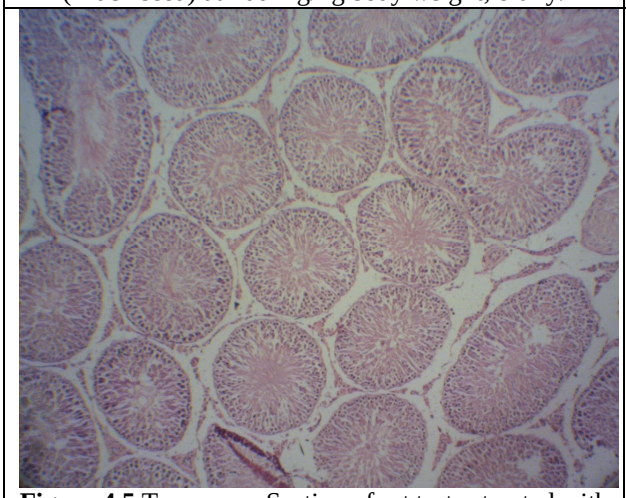


Figure 4.5 Transverse Section of rat testes treated with aqueous extract of *Mucuna cochinchinensis* (Black seed) at 400 mg/kg body weight, orally.

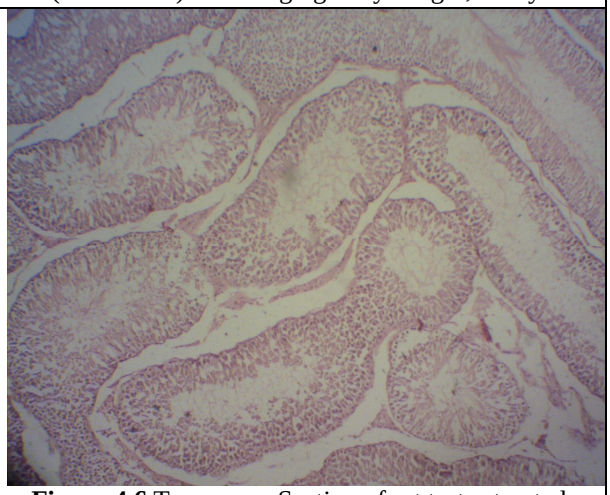


Figure 4.6 Transverse Section of rat testes treated with aqueous extract of *Mucuna cochinchinensis* (White seed) at 100 mg/kg body weight, orally.

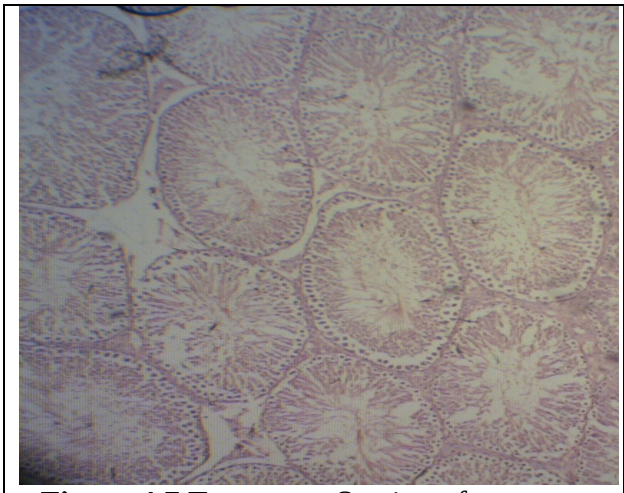


Figure 4.7 Transverse Section of rat testes treated with aqueous extract of *Mucuna cochinchinensis* (White seed) at 200 mg/kg body weight, orally.

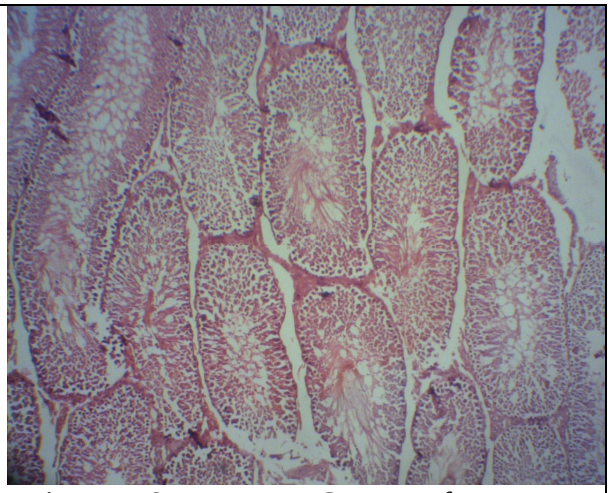


Figure 4.8 Transverse Section of rat testes treated with aqueous extract of *Mucuna cochinchinensis* (White seed) at 400 mg/kg body weight, orally.

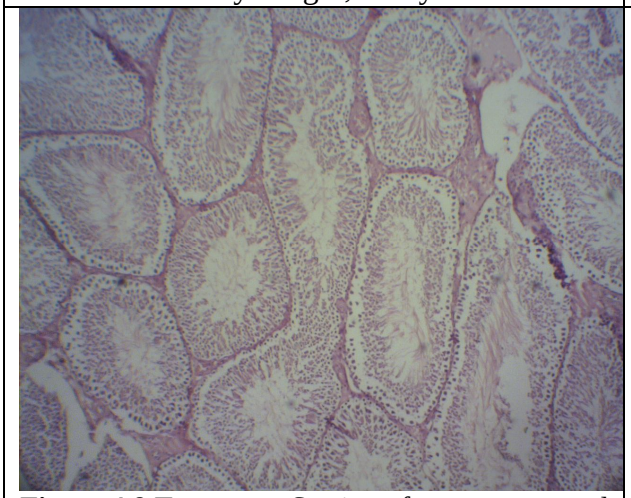


Figure 4.9 Transverse Section of rat testes treated with aqueous extract of *Mucuna pruriens* at 100 mg/kg body weight, orally.

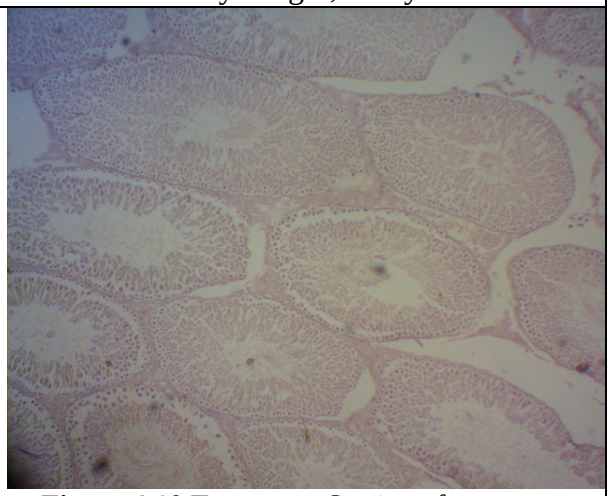


Figure 4.10 Transverse Section of rat testes treated with aqueous extract of *Mucuna pruriens* at 200 mg/kg body weight, orally.

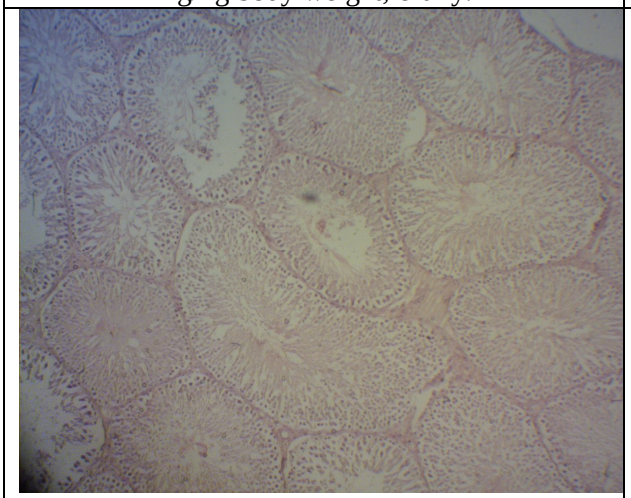


Figure 4.11 Transverse Section of rat testes treated with aqueous extract of *Mucuna pruriens* at 400 mg/kg body weight, orally.

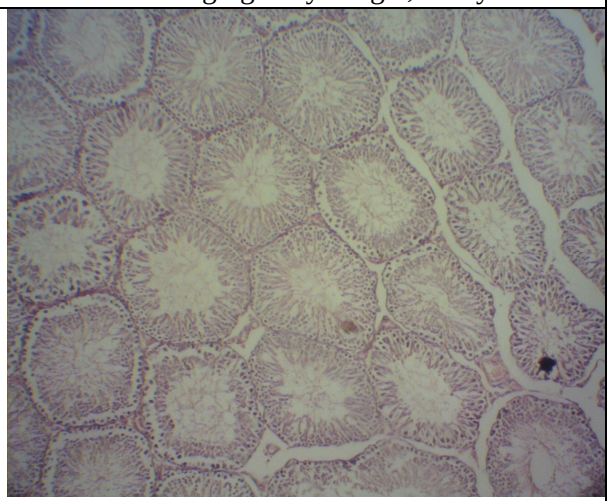


Figure 4.12 Transverse Section of rat testes treated with L-Dopa at 20 mg/kg body weight, orally.

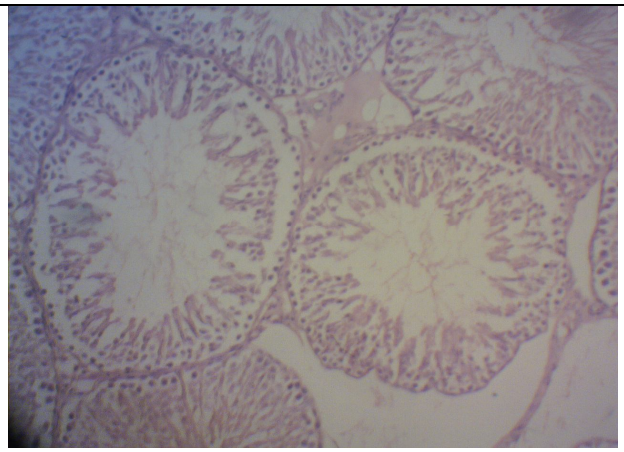


Figure 4.13 Transverse Section of rat testes in Control Group.

Table 4.17 Group mean body weight of rats in gram

Group	Dose mg/kg	Sample		0	7	14	21	28	30
I	0	Control	Mean	260.67	274.33	287.50	301.67	315.33	320.17
			±SD	8.55	9.14	9.48	9.61	9.95	10.89
II	20	Standard Drug (L-dopa)	Mean	261.00	287.67	302.33	317.17	332.00	340.33
			±SD	8.65	6.95	7.37	7.78	7.07	6.06
III	100	*M. cochinchinensis* Black seeds	Mean	258.33	269.83	282.00	294.00	305.67	309.83
			±SD	9.07	7.96	8.07	7.87	8.52	8.40
IV	200		Mean	260.33	272.50	285.50	298.00	310.17	314.67
			±SD	3.88	4.09	4.09	4.15	4.17	3.93
V	400		Mean	258.67	279.17	289.67	309.33	321.17	337.17
			±SD	7.97	5.49	3.56	12.21	6.68	7.86
VI	100	*M. cochinchinensis* White seeds	Mean	261.00	273.33	286.50	299.33	312.33	316.67
			±SD	5.48	5.39	5.68	6.12	6.22	6.65
VII	200		Mean	259.33	271.67	284.00	296.50	308.33	312.00
			±SD	6.65	6.68	6.39	5.92	6.02	5.37
VIII	400		Mean	260.00	271.50	283.83	296.50	307.67	311.17
			±SD	8.20	8.29	8.08	7.97	7.97	7.52
IX	100	*M. pruriens*	Mean	261.33	273.83	286.67	299.83	312.50	318.33
			±SD	5.16	5.04	5.09	5.38	5.89	6.65

X	200		Mean	260.00	273.00	286.33	299.67	312.83	320.17
			±SD	5.80	5.80	5.82	5.82	6.01	6.82
XI	400		Mean	261.33	283.83	294.00	315.17	324.17	331.17
			±SD	5.16	4.54	4.00	5.71	3.19	9.15

Table 4.18. Weight of Testes (Absolute)

Group & Dose (mg/L)	**Control**	**L-dopa**	***M. cochinchinensis* Black seeds**			***M. cochinchinensis* White seeds**			***M. pruriens***		
	G1 0	G2 20	G3 100	G4 200	G5 400	G6 100	G7 200	G8 400	G9 100	G10 200	G11 400
Mean	2.890	3.298	2.901	3.101	3.294	2.827	2.883	2.936	2.899	2.915	3.220
±SD	0.27	0.20	0.18	0.17	0.09	0.34	0.14	0.16	0.23	0.18	0.23

Table 4.19 Weight of Testes (Relative)

Group & Dose (mg/L)	**Control**	**L-dopa**	***M. cochinchinensis* Black seeds**			***M. cochinchinensis* White seeds**			***M. pruriens***		
	G1 0	G2 20	G3 100	G4 200	G5 400	G6 100	G7 200	G8 400	G9 100	G10 200	G11 400
Mean	0.902	0.975	0.912	0.969	0.965	0.894	0.925	0.944	0.936	0.926	0.931
±SD	0.07	0.04	0.06	0.06	0.04	0.12	0.06	0.05	0.08	0.06	0.07

Table 4.20 Weight of Epididymis (Absolute)

Group & Dose (mg/L)	**Control**	**L-dopa**	***M. cochinchinensis* Black seeds**			***M. cochinchinensis* White seeds**			***M. pruriens***		
	G1 0	G2 20	G3 100	G4 200	G5 400	G6 100	G7 200	G8 400	G9 100	G10 200	G11 400
Mean	0.70	0.88	0.74	0.77	0.87	0.69	0.70	0.70	0.70	0.70	0.84
±SD	0.06	0.04	0.03	0.02	0.05	0.05	0.05	0.05	0.05	0.06	0.10

Table 4.21 Weight of Epididymis (Relative)

Group & Dose (mg/L)	**Control**	**L-dopa**	***M. cochinchinensis* Black seeds**			***M. cochinchinensis* White seeds**			***M. pruriens***		
	G1 0	G2 20	G3 100	G4 200	G5 400	G6 100	G7 200	G8 400	G9 100	G10 200	G11 400
Mean	0.220	0.250	0.233	0.240	0.245	0.217	0.226	0.226	0.225	0.224	0.228
±SD	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.01	0.02	0.01

Table 4.22 Sperm count (10^6/ML)

Group & Dose (mg/L)	Control	L-dopa	*M. cochinchinensis* Black seeds			*M. cochinchinensis* White seeds			*M. pruriens*		
	G1 0	G2 20	G3 100	G4 200	G5 400	G6 100	G7 200	G8 400	G9 100	G10 200	G11 400
Mean	20.57	28.83	20.73	21.77	26.48	19.57	20.20	20.27	19.72	20.37	26.00
±SD	1.45	1.96	2.97	2.11	2.99	2.07	2.01	2.13	1.85	2.64	2.15

Table 4.23 Sperm motility (%)

Group & Dose (mg/L)	Control	L-dopa	*M. cochinchinensis* Black seeds			*M. cochinchinensis* White seeds			*M. pruriens*		
	G1 0	G2 20	G3 100	G4 200	G5 400	G6 100	G7 200	G8 400	G9 100	G10 200	G11 400
Mean	81.92	90.58	78.92	81.58	89.67	69.92	73.33	80.58	80.67	77.75	88.50
±SD	3.47	4.16	5.94	6.09	2.09	5.38	6.62	2.92	3.19	5.39	2.74

Table 4.24 Sperm morphology (%)

Group & Dose (mg/L)	Control	L-dopa	*M. cochinchinensis* Black seeds			*M. cochinchinensis* White seeds			*M. pruriens*		
	G1 0	G2 20	G3 100	G4 200	G5 400	G6 100	G7 200	G8 400	G9 100	G10 200	G11 400
Mean	77.57	85.50	75.60	79.80	84.90	69.83	72.20	75.33	70.13	73.57	84.00
±SD	2.32	2.51	5.11	3.50	2.20	3.79	5.53	5.87	5.24	4.35	0.89

Table 4.25 Summary of Necropsy findings Male Rats

	Control	L-dopa	*M. cochinchinensis* Black seeds			*M. cochinchinensis* White seeds			*M. pruriens*		
Group & Dose (mg/kg)	G1 0	G2 20	G3 100	G4 200	G5 400	G6 100	G7 200	G8 400	G9 100	G10 200	G11 400
Necropsy finding	Incidence **(No. of Animals with findings/ initial no. of Animals)**										
No abnormality detected	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6

4.17 Acute toxicity:

Group G1 (Vehicle control) : 0 mg/kg body weight

Mortality:

Distilled water, when tested on three female rats at the dose volume of 10 ml/kg did not cause any mortality on the day of dosing and during the observation period of 14 days thereafter.

Clinical Signs:

Following administration of the vehicle, no toxic signs were observed in all the three rats on the day of dosing and during the 14 days observation period (Table).

Body weights:

The body weight gain of rats was not found to be adversely affected during the 14 days observation period.

Necropsy:

No gross pathological alterations were encountered in any of the female rats when sacrificed at termination of the study.

Group G2: 2000 mg/kg body weight

Mortality:

Mucuna cochinchinensis (Black seed), *Mucuna cochichinensis* (White seed) and *Mucuna pruriens* when tested on three female rats separately at the dose level of 2000 mg/kg body weight did not caused any mortality on the day of dosing and during the observation period of 14 days thereafter .

Clinical Signs:

Following administration of the test article, no evident toxic signs were observed in all the three treated rats on the day of dosing and during the observation period of 14 days.

Body weights:

The body weight gain of all the treated rats was not found to be adversely affected during the 14 days observation period, following dosing .

Necropsy:

No gross pathological alterations were encountered in any of the female rats when sacrificed at termination of the study .

Group G3: 2000 mg/kg body weight

Mortality:

Mucuna cochinchinensis (Black seed), *Mucuna cochichinensis* (White seed) and *Mucuna pruriens* when further tested on three more female rats separately at the dose level of 2000 mg/kg body weight did not caused any mortality on the day of dosing and during the observation period of 14 days thereafter .

Clinical Signs:

Following administration of the test article, no evident toxic signs were observed in all the three treated rats on the day of dosing and during the observation period of 14 days.

Body weights:

The body weight gain of treated rats was not adversely affected following dosing and during the 14 days observation period (Table 4.26).

Necropsy:

No gross pathological alterations were encountered in any of the female rats when sacrificed at termination of the study.

Table 4.26 Body weight data of rats (g) treated with *M. cochinchinensis* (Black seed)

Group	Dose (mg/kg)	Animal ID	Day 0	Day 7	% Gain	Day 15	% Gain
Gr-1 (Vehicle Control)	0	160301	172	184	6.98	197	14.53
		160302	168	181	7.74	195	16.07
		160303	176	189	7.39	203	15.34
		Mean	172.00	184.67	7.37	198.33	15.32
		± SD	4.00	4.04	0.38	4.16	0.77
		N	3	3	-	3	-
Gr-2	2000	160304	176	188	6.82	200	13.64
		160305	170	183	7.65	196	15.29
		160306	174	186	6.90	197	13.22
		Mean	173.33	185.67	7.12	197.67	14.05
		± SD	3.06	2.52	0.46	2.08	1.10
		N	3	3	-	3	-
Gr-3	2000	160307	168	181	7.74	193	14.88
		160308	174	186	6.90	199	14.37
		160309	178	191	7.30	204	14.61
		Mean	173.33	186.00	7.31	198.67	14.62
		± SD	5.03	5.00	0.42	5.51	0.26
		N	3	3	-	3	-

Table 4.27 Body weight data of rats (g) treated with *M. cochichinensis* (White seed)

Group	Dose (mg/kg)	Animal ID	Day 0	Day 7	% Gain	Day 15	% Gain
Gr-1 Vehicle Control	0	160311	160	173	8.13	185	15.63
		160312	168	180	7.14	193	14.88
		160313	174	187	7.47	200	14.94
		Mean	167.33	180.00	7.58	192.67	15.14
		± SD	7.02	7.00	0.50	7.51	6.86
		N	3	3	-	3	-
Gr-2	2000	160314	162	174	7.41	186	14.81
		160315	170	183	7.65	196	15.29
		160316	174	187	7.47	200	14.94
		Mean	168.67	181.33	7.51	194.00	15.02
		± SD	6.11	6.66	0.12	7.21	0.25
		N	3	3	-	3	-
Gr-3	2000	160317	164	177	7.93	190	15.85
		160318	166	178	7.23	191	15.06
		160319	172	184	6.98	196	13.95
		Mean	167.33	179.67	7.38	192.33	14.96
		± SD	4.16	3.79	0.49	3.21	0.95
		N	3	3	-	3	-

Table 4.28 Body weight data of rats (g) when treated with *M. pruriens*

Group	Dose (mg/kg)	Animal ID	Day 0	Day 7	% Gain	Day 15	% Gain
Gr-1 Vehicle Control	0	160321	152	165	8.55	178	17.11
		160322	158	170	7.59	182	15.19
		160323	166	178	7.23	191	15.06
		Mean	158.67	171.00	7.79	183.67	15.76
		± SD	7.02	6.56	-	6.66	-
		N	3	3	-	3	-
Gr-2	2000	160324	150	162	8.00	175	16.67
		160325	162	175	8.02	187	15.43
		160326	168	180	7.14	192	14.29
		Mean	160.00	172.33	7.72	184.67	15.46
		± SD	9.17	9.29	-	8.74	-
		N	3	3	-	3	-
Gr-3	2000	160327	150	163	8.67	175	16.67
		160328	162	175	8.02	188	16.05
		160329	170	182	7.06	195	14.71
		Mean	160.67	173.33	7.92	186.00	15.81
		± SD	10.07	9.61	-	10.15	-
		N	3	3	-	3	-

4.18 Sub-acute Toxicity:

Mortality:

There was no incidence of treatment related mortality among the male and female rats exposed to the aqueous extract of *Mucuna cochinchinensis* (Black seed) at and up to the dose of 1000 mg/kg body wt. All male and female rats from control and treatment groups have survived throughout the period of study.

Clinical Signs:

General and detailed clinical examinations

Treatment of rats with aqueous extract of *Mucuna cochinchinensis* (Black seed) up to the dose of 1000 mg/kg did not cause any noticeable abnormal clinical signs in either sex.

Neurological Examinations:

The neurological examinations and functional observations made on the fourth week of the study did not reveal any observable and treatment related incidences of clinical abnormalities. There were no findings that are indicative of a neurotoxic potential of the aqueous extract of *Mucuna cochinchinensis* (Black seed), were encountered during these examinations.

Body Weights:

Aqueous extract of *Mucuna cochinchinensis* (Black seed) did not induce any remarkable and significant alteration in the body weights of the male and female rats treated at and up to the dose of 1000mg/kg.

The group mean body weights of male and female rats treated with aqueous extract of *Mucuna cochinchinensis* (Black seed) at and up to the dose of 1000mg/kg on termination of study, did not differ significantly from those of the controls during the 28 day treatment period. During the reversal period, the groups mean body weight of male and female rats treated with aqueous extract of *Mucuna cochinchinensis* (Black seed) at the dose of 1000 mg/kg showed normal increase in the body weights during reversal period of the 14 days.

Food Consumption:

The values of average daily food consumption by male and female rats exposed to aqueous extract of *Mucuna cochinchinensis* (Black seed) at and up to the dose of 1000mg/kg were found to be comparable to those of the control groups. The average daily food consumption per rat per day, computed over the period of 4 weeks, by male rats receiving aqueous extract of *Mucuna cochinchinensis* (Black seed) at

250mg/kg, 500mg/kg and 1000mg/kg was 106%, 97% and 98% respectively of that by control rats. Similarly, the average daily food consumption by female rats receiving the aqueous extract of *Mucuna cochinchinensis* (Black seed) at above dose levels was 94%, 105% and 104% respectively of that by control rats. After cessation of treatment, during the recovery period too, the values of daily food intake by the intermediate dose group rats were found to be gradually increasing as during the treatment.

Organ Weights:

The group mean values of absolute and relative weights of liver, kidneys, adrenals, testes / ovaries, spleen, brain and heart of male and female rats treated at and up to 1000mg/kg were found to be comparable to those of the respective control group of rats at termination of the treatment and reversal periods in this study.

Haematology:

The group mean values of haematological parameters viz. haemoglobin, haematocrit (PCV) and total erythrocyte counts, differential leucocyte counts, erythrocyte indices, platelet count and clotting time of male and female rats exposed to aqueous extract of *Mucuna cochinchinensis* (Black seed) at and up to the level of 1000mg/kg did not reveal any treatment related effect on termination of the study and reversal periods in this study.

Clinical Chemistry:

Treatment of male and female rats with aqueous extract of *Mucuna cochinchinensis* (Black seed) at and up to the dose of 1000mg/kg did not exhibit any remarkable treatment effect / change, on their plasma biochemistry related parameters. With some exceptions, like group mean plasma levels of total protein, albumin, and alanine aminotransferase (ALT), globulin, bilirubin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose, urea nitrogen, creatinine, total cholesterol, triglycerides, calcium phosphorous, sodium and potassium in male and female rats treated with aqueous extract of *Mucuna cochinchinensis* (Black seed) at and up to the dose of 1000mg/kg did not differ significantly from those of the respective control groups or did not show any treatment effect, as evident at end of the treatment and reversal periods of the study.

Urinalysis:

Treatment of male and female rats with aqueous extract of *Mucuna cochinchinensis* (Black seed) at and up to the dose of 1000mg/kg did not exhibit any

remarkable effect on their urine related parameters. The qualitative parameters of urine evaluated for all rats in this study and the microscopic examination of their urine sediment did not reveal any noticeable, treatment related alterations.

Necropsy:

Aqueous extract of *Mucuna cochinchinensis* (Black seed), up to the level of 1000mg/kg, did not induce / cause any remarkable and treatment related gross pathological changes in any of the organs / tissues of treated rats, as evident during the detailed necropsy carried out on termination of the study.

Conclusion:

Aqueous extract of *Mucuna cochinchinensis* (Black seed), after daily oral administration at the doses of 250mg/kg,500mg/kg, and 1000mg/kg, to different groups of five Sprague Dawley rats per sex for 28 days caused:

- No treatment related mortality in male and female rats, at different doses and up to the dose of 1000mg/kg.
- No incidence of any noticeable abnormality in respect of clinical signs in either sex, at different doses and up to the dose of 1000mg/kg.
- No significant effect on the body weights of male and female rats, at and up to the dose of 1000mg/kg.
- No effect on the absolute and relative organ weights of male and female rats, at different doses and up to the dose of 1000mg/kg.
- No toxicologically significant effect on the haematological parameters of male and female rats treated at different doses and up to the dose of 1000mg/kg.
- No neurotoxicity, at different doses and up to the dose of 1000mg/kg.
- No effect on the biochemical parameters of male and female rats treated at different doses and up to the dose of 1000mg/kg.
- No effect on the urinalysis parameters of male and female rats treated at different doses and up to the dose of 1000mg/kg.

Results of the study indicated that the studied aqueous extract of *Mucuna cochinchinensis* (Black seed) doesn't have any adverse effect on general health, growth, behavioural, neurological, haematological, clinical chemistry and urinalysis parameters, organ weights and gross and microscopic appearance of the tissues / organs of the rats treated at different doses and up to the dose level of 1000mg/kg body weight.

Based on these findings of this study, the no observed adverse effect level (NOAEL) of aqueous extract of *Mucuna cochinchinensis* (Black seed) in rats, following oral administration for 28 days at and up to 1000 mg/kg body weight.

Table 4.29 Summary of Neurological examination (Male)

Group	G1	G2-R	G3	G4	G5	G6-R
Dose (mg/kg)	**0**	**0**	**250**	**500**	**1000**	**1000**
Findings	Incidence (No. of animals with findings)					
Nos. Examined	5	5	5	5	5	5
Sensory Reactivity	No abnormality detected					
Motor Activity	No abnormality detected					
Grip Strength	No abnormality detected					

Measured parameters

Group		G1	G2-R	G3	G4	G5	G6-R
Urination, frequency	Mean	0.60	0.63	0.40	0.60	0.60	0.63
	± SD	0.55	0.77	0.55	0.55	0.55	0.71
	n	5	5	5	5	5	5
Defecation, frequency	Mean	0.40	0.55	0.80	0.60	0.80	0.76
	± SD	0.55	0.66	0.84	0.89	0.84	0.81
	n	5	5	5	5	5	5
Rearing, frequency	Mean	1.80	1.95	2.00	2.20	2.60	2.00
	± SD	0.84	0.83	0.71	0.84	0.89	0.87
	n	5	5	5	5	5	5
Landing foot splay (cm)	Mean	6.80	6.35	6.44	6.52	6.66	6.54
	± SD	0.37	0.43	0.60	0.49	0.34	0.45
	n	5	5	5	5	5	5

Note: Frequency for measured parameters pertains to number of occurrences in 3 minutes.

Table 4.30 Summary of Neurological Examination (Female)

Group	G1	G2-R	G3	G4	G5	G6-R
Dose (mg/kg)	**0**	**0**	**250**	**500**	**1000**	**1000**
Findings	Incidence (No. of animals with findings)					
Nos. Examined	5	5	5	5	5	5
Sensory Reactivity	No abnormality detected					
Motor Activity	No abnormality detected					
Grip Strength	No abnormality detected					

Measured Parameters

Group		G1	G2-R	G3	G4	G5	G6-R
Urination, frequency	Mean	0.40	0.45	0.20	0.40	0.40	0.35
	± SD	0.71	0.65	0.00	0.00	0.00	0.11
	n	5	5	5	5	5	5
Defecation, frequency	Mean	0.40	0.47	0.40	0.40	1.20	1.35
	± SD	0.00	0.16	0.00	0.71	0.00	0.25
	n	5	5	5	5	5	5
Rearing, frequency	Mean	2.20	2.25	2.40	2.40	2.20	2.33
	± SD	1.41	1.53	1.41	0.71	1.41	1.53
	n	5	5	5	5	5	5
Landing foot splay (cm)	Mean	6.74	6.25	6.74	6.92	6.64	6.57
	± SD	0.21	0.29	0.42	0.28	0.00	0.27
	n	5	5	5	5	5	5

Note: Frequency for measured parameters pertains to number of occurrences in 3 minutes.

Table 4.31 Summary of Body Weights (g) in male rats

Groups	Dose (mg/kg)		Study week - Treatment Period						
			0	**1**	**2**	**3**	**4**	**5**	**6**
G1	**0**	**Mean**	178.80	189.60	201.20	212.60	223.40	-	-
		± SD	5.97	4.88	6.53	5.94	6.99	-	-
		N	5	5	5	5	5	-	-
G2-R	**0**	**Mean**	178.00	188.20	198.20	208.40	219.60	229.60	240.80
		± SD	5.92	5.89	4.76	4.51	4.88	6.62	7.40
		N	5	5	5	5	5	5	5
G3	**250**	**Mean**	178.20	189.20	200.60	210.80	222.20	-	-
		± SD	3.83	4.02	4.34	4.87	6.18	-	-
		N	5	5	5	5	5	-	-
G4	**500**	**Mean**	178.00	189.40	201.00	211.40	222.20	-	-
		± SD	3.87	3.21	3.94	4.39	5.36	-	-
		N	5	5	5	5	5	-	-
G5	**1000**	**Mean**	179.00	188.60	198.40	209.00	218.60	-	-
		± SD	7.84	8.38	8.14	7.55	8.14	-	-
		N	5	5	5	5	5	-	-
G6-R	**1000**	**Mean**	179.80	190.00	200.20	210.20	221.20	231.40	242.40
		± SD	6.30	6.44	5.67	6.76	7.76	8.08	9.10
		N	5	5	5	5	5	5	5

Table 4.32 Summary of Body Weights (g) in Female rats

Groups	Dose (mg/ kg)		Study week - Treatment Period						
			0	**1**	**2**	**3**	**4**	**5**	**6**
G1	**0**	**Mean**	169.80	180.80	190.40	202.60	215.00	-	-
		± SD	4.21	5.81	5.41	4.93	4.58	-	-
		N	5	5	5	5	5	-	-
G2-R	**0**	**Mean**	168.80	179.80	190.20	200.40	210.80	221.20	231.80
		± SD	6.14	6.76	6.98	7.70	6.98	7.26	8.35
		N	5	5	5	5	5	5	5
G3	**250**	**Mean**	169.00	179.80	192.00	202.00	213.60	-	-
		± SD	5.24	6.50	7.91	7.28	8.35	-	-
		N	5	5	5	5	5	-	-
G4	**500**	**Mean**	169.80	180.40	192.40	203.80	214.40	-	-
		± SD	5.17	5.81	5.59	5.45	6.54	-	-
		N	5	5	5	5	5	-	-
G5	**1000**	**Mean**	170.40	181.00	190.80	200.80	213.40	-	-
		± SD	4.67	3.74	2.59	2.05	2.88	-	-
		N	5	5	5	5	5	-	-
G6-R	**1000**	**Mean**	168.80	178.40	188.80	199.60	210.40	221.20	232.00
		± SD	4.55	5.22	5.40	4.83	5.68	5.22	6.32
		N	5	5	5	5	5	5	5

Table 4.33 Summary of food consumption (g) in male rats

Groups	Dose (mg/kg)	Study week - Treatment Period						Study Average
		1	2	3	4	5	6	Week 1-4
G1 and G2	0	14.2	12.5	13.2	12.4	12.9	13.5	13.08
G3	250	13.5	12.8	12.9	12.6	-	-	12.95
	% of Control	95	102	98	102	-	-	99
G4	500	12.6	13.2	12.5	13.1	-	-	12.85
	% of Control	89	106	95	106	-	-	98
G5 and G6	1000	14.3	13.2	12.8	12.2	13.1	12.7	13.13
	% of Control	101	106	97	98	99	103	100

Table 4.34 Summary of food consumption (g) in female rats

Groups	Dose (mg/kg)	Study week – Treatment Period						Study Average
		1	2	3	4	5	6	Week 1-4
G1 and G2	0	12.8	13.1	12.6	12.9	13.4	12.7	12.85
G3	250	12.4	12.6	13.2	12.8	-	-	12.75
	% of Control	97	96	105	99	-	-	99
G4	500	13.2	12.9	11.6	13.4	-	-	12.78
	% of Control	103	98	92	104	-	-	99
G5 and G6	1000	12.9	12.3	13.2	13.4	13.1	13.4	12.95
	% of Control	101	94	105	104	100	104	101

Table 4.35 Summary of Absolute organ weight (g) in male rats

Group	Dose (mg/kg)		Adrenal	Testes	Spleen	Heart	Thymus	Brain	Epididymis	Kidneys	Liver
G1	**0**	**Mean**	0.072	3.020	1.675	1.707	0.732	2.906	1.455	2.909	9.209
		± SD	0.010	0.232	0.279	0.231	0.090	0.250	0.189	0.213	0.950
		N	5	5	5	5	5	5	5	5	5
G2-R	**0**	**Mean**	0.074	2.995	1.551	1.770	0.758	2.893	1.495	2.928	9.064
		± SD	0.008	0.106	0.101	0.218	0.064	0.145	0.112	0.186	0.928
		N	5	5	5	5	5	5	5	5	5
G3	**250**	**Mean**	0.074	3.039	1.658	1.782	0.768	2.910	1.453	2.904	9.551
		± SD	0.009	0.147	0.250	0.387	0.084	0.217	0.162	0.226	0.900
		N	5	5	5	5	5	5	5	5	5
G4	**500**	**Mean**	0.073	2.918	1.687	1.948	0.792	2.993	1.517	2.937	9.666
		± SD	0.011	0.403	0.218	0.163	0.106	0.354	0.180	0.226	1.071
		N	5	5	5	5	5	5	5	5	5
G5	**1000**	**Mean**	0.074	2.986	1.667	2.022	0.741	2.953	1.534	2.846	9.520
		± SD	0.005	0.341	0.224	0.111	0.100	0.295	0.245	0.268	0.857
		N	5	5	5	5	5	5	5	5	5
G6-R	**1000**	**Mean**	0.075	2.836	1.621	1.961	0.719	2.832	1.646	2.755	9.505
		± SD	0.008	0.150	0.123	0.097	0.132	0.172	0.165	0.157	0.942
		N	5	5	5	5	5	5	5	5	5

Table 4.36 Summary of Absolute organ Weight (g) in female rats

Group	Dose (mg/kg)		Adrenal	Ovaries	Spleen	Heart	Thymus	Brain	Uterus	Kidneys	Liver
G1	**0**	**Mean**	0.068	0.086	1.596	1.676	0.764	2.756	0.736	2.891	8.754
		± SD	0.006	0.006	0.328	0.276	0.071	0.536	0.087	0.270	1.491
		N	5	5	5	5	5	5	5	5	5
G2-R	**0**	**Mean**	0.069	0.085	1.633	1.723	0.762	2.708	0.731	2.828	8.620
		± SD	0.010	0.008	0.186	0.179	0.074	0.350	0.087	0.159	1.425
		N	5	5	5	5	5	5	5	5	5
G3	**250**	**Mean**	0.068	0.087	1.513	1.740	0.747	2.994	0.786	2.779	8.900
		± SD	0.004	0.006	0.284	0.398	0.082	0.206	0.148	0.312	1.060
		N	5	5	5	5	5	5	5	5	5
G4	**500**	**Mean**	0.069	0.084	1.531	1.506	0.737	2.773	0.745	2.916	9.397
		± SD	0.009	0.008	0.305	0.418	0.093	0.232	0.103	0.235	1.329
		N	5	5	5	5	5	5	5	5	5
G5	**1000**	**Mean**	0.068	0.084	1.500	1.506	0.760	2.781	0.761	2.823	9.119
		± SD	0.008	0.007	0.184	0.248	0.108	0.296	0.092	0.304	1.147
		N	5	5	5	5	5	5	5	5	5
G6-R	**1000**	**Mean**	0.069	0.086	1.507	1.661	0.764	2.819	0.764	2.936	8.958
		± SD	0.009	0.006	0.155	0.154	0.089	0.312	0.125	0.142	1.146
		N	5	5	5	5	5	5	5	5	5

Table 4.37 Summary of Relative organ weights (%) in male rats

Group	Dose (mg/kg)		Adrenal	Testes	Spleen	Heart	Thymus	Brain	Epididymis	Kidneys	Liver
G1	0	Mean	0.032	1.351	0.751	0.763	0.328	1.301	0.653	1.302	4.132
		± SD	0.004	0.082	0.130	0.092	0.042	0.102	0.091	0.092	0.510
		N	5	5	5	5	5	5	5	5	5
G2-R	0	Mean	0.033	1.341	0.694	0.791	0.339	1.297	0.670	1.312	4.058
		± SD	0.004	0.055	0.041	0.081	0.021	0.092	0.059	0.100	0.400
		N	5	5	5	5	5	5	5	5	5
G3	250	Mean	0.055	1.572	1.385	1.872	0.542	1.744	1.035	1.757	6.404
		± SD	0.052	0.455	1.490	2.457	0.455	0.987	0.886	1.062	4.567
		N	5	5	5	5	5	5	5	5	5
G4	500	Mean	0.033	1.314	0.760	0.876	0.357	1.346	0.683	1.322	4.358
		± SD	0.004	0.189	0.102	0.063	0.050	0.146	0.082	0.105	0.556
		N	5	5	5	5	5	5	5	5	5
G5	1000	Mean	0.034	1.367	0.764	0.926	0.339	1.352	0.703	1.303	4.367
		± SD	0.003	0.159	0.116	0.061	0.044	0.140	0.121	0.127	0.515
		N	5	5	5	5	5	5	5	5	5
G6-R	1000	Mean	0.034	1.298	0.743	0.898	0.330	1.297	0.753	1.262	4.347
		± SD	0.003	0.066	0.065	0.058	0.063	0.089	0.077	0.097	0.380
		N	5	5	5	5	5	5	5	5	5

Table 4.38 Summary of Relative organ weight (%) in female rats

Group	Dose (mg/kg)		Adrenal	Ovaries	Spleen	Heart	Thymus	Brain	Uterus	Kidneys	Liver
G1	**0**	**Mean**	0.032	0.040	0.745	0.780	0.355	1.284	0.343	1.347	4.081
		± SD	0.003	0.004	0.166	0.127	0.033	0.261	0.045	0.155	0.754
		N	5	5	5	5	5	5	5	5	5
G2-R	**0**	**Mean**	0.032	0.039	0.761	0.802	0.355	1.261	0.340	1.316	4.001
		± SD	0.004	0.004	0.100	0.088	0.040	0.175	0.037	0.095	0.592
		N	5	5	5	5	5	5	5	5	5
G3	**250**	**Mean**	0.032	0.041	0.706	0.814	0.349	1.402	0.367	1.300	4.175
		± SD	0.003	0.002	0.113	0.179	0.031	0.084	0.061	0.127	0.539
		N	5	5	5	5	5	5	5	5	5
G4	**500**	**Mean**	0.032	0.039	0.712	0.702	0.344	1.295	0.348	1.363	4.392
		± SD	0.004	0.004	0.130	0.198	0.042	0.122	0.055	0.140	0.689
		N	5	5	5	5	5	5	5	5	5
G5	**1000**	**Mean**	0.032	0.039	0.703	0.706	0.356	1.305	0.357	1.322	4.272
		± SD	0.004	0.003	0.089	0.118	0.052	0.155	0.045	0.128	0.527
		N	5	5	5	5	5	5	5	5	5
G6-R	**1000**	**Mean**	0.032	0.040	0.707	0.779	0.358	1.323	0.358	1.376	4.201
		± SD	0.005	0.003	0.075	0.075	0.046	0.162	0.058	0.075	0.564
		N	5	5	5	5	5	5	5	5	5

Table 4.39 Group Mean Haematology in male rats.

Group	Dose (mg/kg)		Hb (g/dl)	PCV (%)	Total RBC ($x10^6$/ cmm)	RBC Indices			Total WBC ($x10^3$/ cmm)	Differential WBC (%)				Platelets ($x10^3$/ cmm)	Clotting Time (sec)
						MCH (pg)	MCV (fl)	MCHC (g/dl)		N	L	E	M		
G1	0	Mean	12.82	38.70	8.18	15.78	47.58	33.33	12.20	28.80	67.20	2.20	1.80	830.80	98.00
		± SD	0.99	2.76	0.60	2.09	5.42	4.21	1.19	4.21	4.27	1.30	0.84	80.28	5.79
		N	5	5	5	5	5	5	5	5	5	5	5	5	5
G2-R	0	Mean	12.74	39.24	8.26	15.49	47.60	32.52	12.74	27.00	69.60	2.00	1.40	834.20	99.20
		± SD	0.62	1.53	0.51	1.46	2.35	2.24	0.65	4.18	4.34	1.00	1.14	52.94	3.03
		N	5	5	5	5	5	5	5	5	5	5	5	5	5
G3	250	Mean	12.34	38.84	8.20	15.15	47.90	31.93	12.28	29.00	67.40	2.20	1.40	840.60	97.40
		± SD	0.81	2.30	0.96	1.27	6.46	3.78	1.06	5.15	4.72	1.30	1.14	59.02	5.86
		N	5	5	5	5	5	5	5	5	5	5	5	5	5
G4	500	Mean	12.52	40.10	8.34	15.17	48.36	31.27	12.34	30.20	66.00	2.40	1.40	832.40	98.60
		± SD	0.82	2.02	0.92	2.12	3.25	2.36	1.24	3.96	3.00	1.14	1.14	66.26	10.78
		N	5	5	5	5	5	5	5	5	5	5	5	5	5
G5	1000	Mean	12.64	38.32	8.32	15.26	46.43	33.17	12.40	29.00	67.20	2.40	1.40	832.60	100.20
		± SD	1.00	2.69	0.86	1.26	5.78	4.15	1.22	4.85	3.35	1.14	1.14	78.00	3.27
		N	5	5	5	5	5	5	5	5	5	5	5	5	5
G6-R	1000	Mean	12.40	40.08	8.20	15.28	49.37	30.94	12.72	30.00	67.40	1.80	0.80	836.80	98.20
		± SD	0.69	1.10	0.85	2.06	6.03	1.65	0.76	2.24	3.71	1.64	0.84	66.24	4.44
		N	5	5	5	5	5	5	5	5	5	5	5	5	5

Table 4.40 Group Mean Haematology in female rats.

Group	Dose (mg/kg)		Hb (g/dl)	PCV (%)	Total RBC ($x10^6$/ cmm)	RBC Indices			Total WBC ($x10^3$/ cmm)	Differential WBC (%)				Platelets ($x10^3$/ cmm)	Clotting Time (sec)
						MCH (pg)	MCV (fl)	MCHC (g/dl)		N	L	E	M		
G1	0	Mean	12.54	39.20	8.26	15.23	47.50	32.05	12.22	27.60	68.20	2.40	1.80	840.20	97.60
		± SD	1.04	2.43	0.57	1.49	1.86	2.75	1.27	5.37	4.02	1.14	0.84	64.08	6.54
		N	5	5	5	5	5	5	5	5	5	5	5	5	5
G2-R	0	Mean	12.74	39.24	8.50	15.04	46.60	32.59	12.54	28.20	68.00	2.40	1.40	839.60	97.00
		± SD	0.66	2.17	0.76	0.85	6.37	3.01	0.85	3.03	1.58	1.34	1.14	72.05	5.92
		N	5	5	5	5	5	5	5	5	5	5	5	5	5
G3	250	Mean	12.64	38.94	8.30	15.32	47.12	32.59	12.08	28.00	68.40	2.20	1.40	843.60	96.00
		± SD	0.69	2.42	0.62	1.65	4.56	3.07	0.99	4.47	4.72	1.30	1.14	45.42	9.30
		N	5	5	5	5	5	5	5	5	5	5	5	5	5
G4	500	Mean	12.38	38.98	8.10	15.35	48.56	31.83	12.16	26.80	69.60	2.00	1.60	845.00	96.00
		± SD	0.69	1.98	0.72	1.17	6.35	2.42	1.29	3.03	4.04	1.22	1.14	55.63	12.00
		N	5	5	5	5	5	5	5	5	5	5	5	5	5
G5	1000	Mean	12.44	39.30	8.18	15.29	48.39	31.67	12.32	28.40	67.40	2.40	1.80	842.80	94.20
		± SD	0.70	1.70	0.66	1.62	5.69	1.59	1.12	3.21	2.97	1.14	0.84	59.48	9.01
		N	5	5	5	5	5	5	5	5	5	5	5	5	5
G6-R	1000	Mean	12.64	38.90	8.44	15.06	46.44	32.60	12.66	28.00	68.20	1.80	2.00	838.80	94.60
		± SD	0.81	1.98	0.87	1.33	4.72	3.27	0.59	1.41	1.92	1.30	1.00	65.20	5.03
		N	5	5	5	5	5	5	5	5	5	5	5	5	5

Table 4.41 Group mean clinical chemistry in male rats.

Group	Dose (mg/kg)		Bili (mg/dl)	TP (g/dl)	Albu (g/dl)	Glob (g/dl)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Gluc (mg/dl)	TC (mg/dl)	BUN (mg/dl)	Creat. (mg/dl)	Ca (mg/dl)	P (mg/dl)	TG (mg/dl)	Na (mmol/L)	K (mmol/L)
G1	0	Mean	0.60	6.32	4.38	1.94	78.40	103.60	162.40	105.40	158.60	13.10	0.67	11.62	6.58	130.20	157.80	7.38
		± SD	0.12	1.07	1.00	0.82	8.20	7.20	5.90	7.57	7.16	1.66	0.11	0.89	0.72	4.32	8.41	1.59
		N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
G2-R	0	Mean	0.59	6.20	4.18	2.02	77.80	101.40	162.00	102.80	157.00	13.84	0.66	11.62	6.74	130.40	157.00	7.35
		± SD	0.07	0.60	0.91	1.10	9.15	2.88	5.15	4.82	4.90	0.88	0.08	0.75	0.61	2.70	5.34	0.86
		N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
G3	250	Mean	0.61	6.24	4.22	2.02	75.40	100.60	159.00	102.00	156.20	12.62	0.72	11.70	7.10	130.00	155.40	7.33
		± SD	0.08	1.02	0.58	1.00	7.64	4.22	5.24	5.70	8.32	0.59	0.14	0.35	1.24	2.92	5.68	1.55
		N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
G4	500	Mean	0.60	6.98	4.32	2.66	77.40	104.40	159.00	103.80	157.60	13.24	0.69	11.82	7.14	132.00	155.80	7.46
		± SD	0.14	0.68	0.88	1.18	9.50	7.77	7.07	7.79	6.02	1.39	0.11	0.94	1.01	6.32	8.17	1.51
		N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
G5	1000	Mean	0.59	6.72	4.16	2.56	74.60	104.00	156.40	104.80	157.00	13.30	0.68	11.84	6.92	131.00	156.00	7.03
		± SD	0.10	0.80	0.90	0.86	6.54	7.91	8.20	5.63	8.72	1.64	0.09	1.15	0.67	5.70	7.91	1.73
		N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
G6-R	1000	Mean	0.58	6.10	4.46	1.64	74.40	102.40	156.80	102.60	155.00	13.70	0.67	11.82	7.10	129.20	156.20	7.94
		± SD	0.10	0.69	1.04	1.35	6.88	4.04	5.31	6.02	6.60	1.02	0.07	1.00	0.58	4.60	5.72	0.82
		N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Table 4.42 Group mean clinical Chemistry female rats.

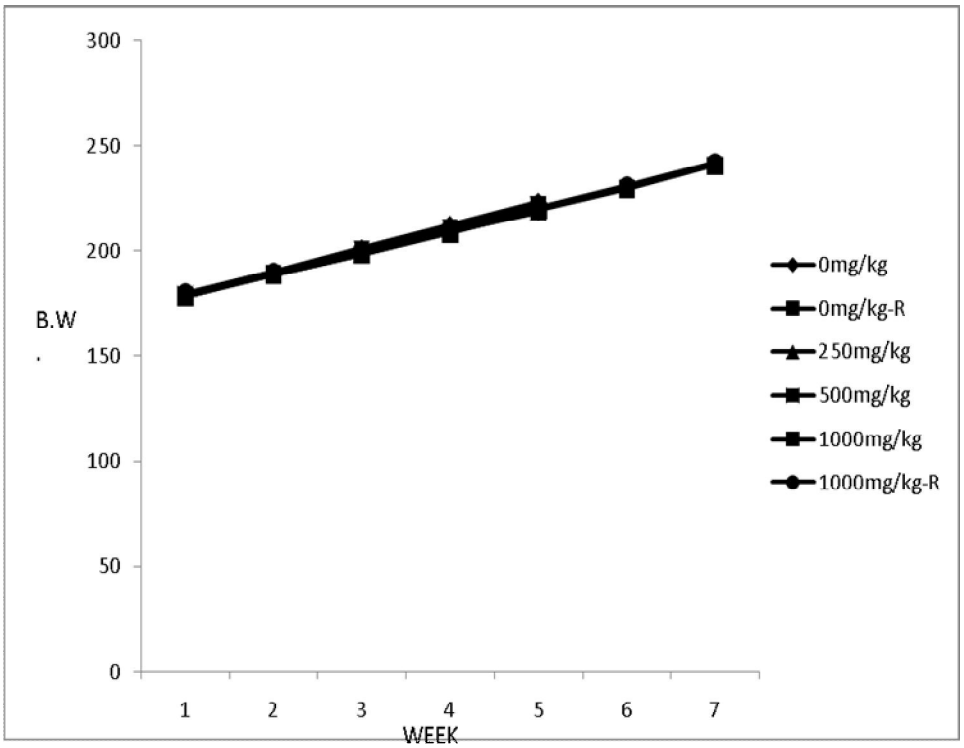
Group	Dose (mg/kg)		Bilirubin (mg/dl)	TP (g/dl)	Albu (g/dl)	Glob (g/dl)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Gluc (mg/dl)	TC (mg/dl)	BUN (mg/dl)	CR. (mg/dl)	Ca (mg/dl)	P (mg/dl)	TG (mg/dl)	Na (mmol/L)	K (mmol/L)
Day 29																		
G1	**0**	**Mean**	0.58	6.12	4.44	1.68	75.20	103.40	158.00	100.80	154.00	13.30	0.67	11.58	6.76	130.20	156.40	6.29
		± SD	0.10	0.79	0.67	0.65	11.45	7.83	6.71	4.76	5.34	1.30	0.12	0.82	0.47	5.17	9.86	1.15
		N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
G2-R	**0**	**Mean**	0.59	6.24	4.64	1.60	74.20	101.00	156.00	102.00	158.40	12.80	0.66	11.50	6.96	128.40	156.20	6.97
		± SD	0.09	0.73	0.63	0.91	7.09	6.60	6.82	5.10	5.81	0.95	0.14	0.69	0.68	2.41	7.29	0.92
		N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
G3	**250**	**Mean**	0.56	6.06	4.12	1.94	76.00	103.20	158.20	102.00	156.80	12.78	0.66	11.82	7.38	133.00	157.00	6.84
		± SD	0.12	0.27	0.93	0.98	10.56	5.97	4.97	6.44	6.69	0.90	0.09	0.81	2.00	4.74	6.32	1.68
		N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
G4	**500**	**Mean**	0.56	5.68	4.62	1.06	76.00	103.00	157.40	100.80	155.40	12.68	0.70	11.36	7.76	129.00	155.80	6.60
		± SD	0.11	0.52	0.90	0.42	9.62	4.36	7.37	8.23	7.09	0.72	0.12	0.98	0.94	4.74	6.53	1.26
		N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
G5	**1000**	**Mean**	0.54	6.38	4.70	1.68	74.60	103.60	159.60	102.40	155.80	12.78	0.71	11.64	7.52	128.60	155.20	6.37
		± SD	0.09	0.83	0.89	1.05	8.73	5.59	7.09	11.55	7.33	1.29	0.15	0.70	1.43	5.13	6.46	0.80
		N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
G6-R	**1000**	**Mean**	0.55	5.88	4.48	1.40	76.00	99.80	161.20	101.20	156.80	12.76	0.70	11.80	8.10	128.40	156.80	7.31
		± SD	0.09	0.49	0.68	0.92	5.57	3.11	7.92	2.68	5.89	0.71	0.10	0.68	0.76	4.04	8.26	1.13
		N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Table 4.43 Summary of Necropsy Findings in male rats

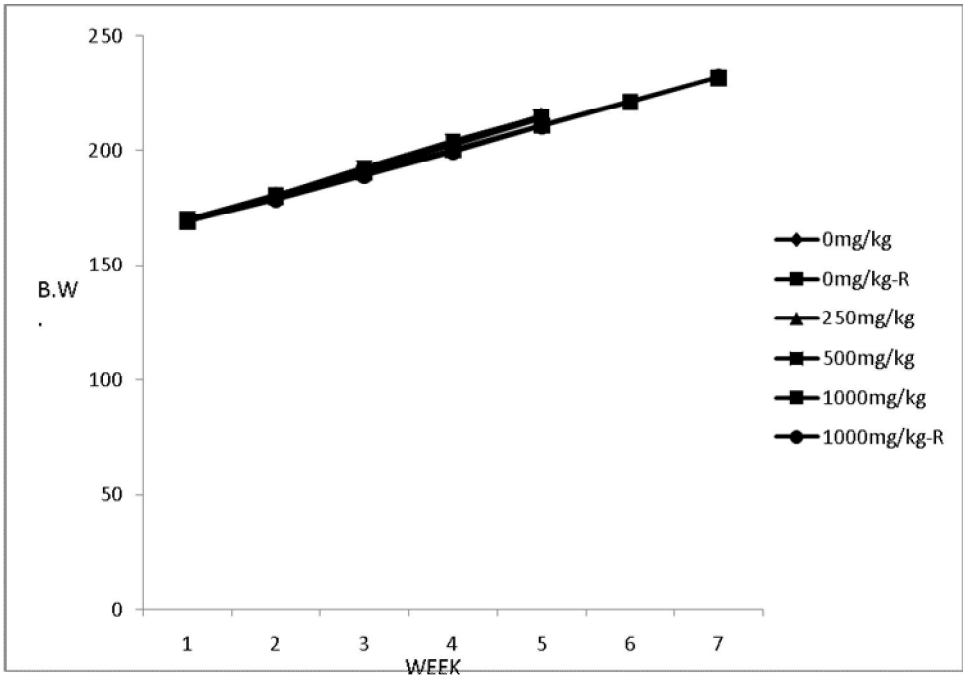
Group & Dose (mg/kg)	**G1 0**	**G2-R 0**	**G3 250**	**G4 500**	**G5 1000**	**G6-R 1000**
Necropsy finding	Incidence (No. of Animals with findings/ initial no. of Animals)					
No abnormality detected	5/5	5/5	5/5	5/5	5/5	5/5

Table 4.44 Summary of Necropsy Findings in female rats

Group & Dose (mg/kg)	**G1 0**	**G2-R 0**	**G3 250**	**G4 500**	**G5 1000**	**G6-R 1000**
Necropsy finding	Incidence (No. of Animals with findings/ initial no. of Animals)					
No abnormality detected	5/5	5/5	5/5	5/5	5/5	5/5



Graph 4.17: Body weight in Male rats



Graph 4.18: Body Weight in Female Rat

Chapter 5: Discussion

Ancient traditional system of Indian medicine, Ayurveda, has uniqueness in providing treatment to humanity. As this system believes in not only treating the diseases, but also has shown various methods and procedures in protecting the health of every healthy individual (Swasthasya swasthya rakshanam). As part of this, Ayurvedic scholars explained Vrishya chikitsa to protect and nourish the body tissues with the target of germinative tissues of individual. As defined by Acharya Vagbhata, Vrishya is a drug or therapy which bestows offspring, immediate feeling of joy, capacity to copulate with women with great vigour like horse and becoming dearer to women thereby is known as vajeekarana (Virilification). (A.S.Ut.50/1-3). By keeping this therapy among eight main branches of Ayurveda, this system has given due importance to have healthy progeny by every individual. 'Kapikachu', a well known Ayurvedic plant drug, mentioned by most of Ayurvedic scholars for its Balya and Vrishya (Spermatogenic) activity. Such hundreds of medicinal plant preparations and uses have mentioned in Ayurveda for treating various diseases of mankind. Even though the rationality of the use of these plant based preparations in diseases is discussed in texts of Ayurveda, the validity of these uses is need to be carried out for better understanding to the present scientific fraternity. In the process of validating, experimental studies with more recent parameters, techniques, provide a better acceptable data in drawing conclusions in respect of efficacy of natural products.

In the Discussion chapter, logical reasoning of observations resulted from this study, by comparing them with the previous related studies is thoroughly done and reported. This chapter helps in interpreting the results, discussing the observations of the study and drawing the conclusions which can provide leads for future researches / investigators in this area.

5.1 Drug Review

Kapikachu is one of the major ingredients of many preparations prescribed by different Ayurvedic scholars for the treatment of many diseases. Literature of Kapikachu is thoroughly reviewed from many ayurvedic classical texts for better

understanding and development of database. The drug review is divided into two parts (based on the period of author) as

1. Samhita Kala, 2. Nighantu kala.

1. Samhita kala: In different Samhitas 47 references of Kapikachu were found. Acharyas like Caraka and Susruta has mentioned many preparations, like Maha Mayura ghrita, Amrita prasha ghrita etc with Kapikachu for the diseases like Unmada, and for the first time Acharya Susruta used Kapikachu for Vajikarana chikitsa.

2. Nighantu kala: As it was told in Rajanighantu, every physician of Ayurveda needs to learn the knowledge of Nighantus, for better understanding of plants / drugs and their uses. Bhavamishra in his Bhavaprakasha Nighantu, used this drug for vata disorders and also used the seed powder of this plant for Vajeekarana purpose. In Sodhala nighantu it was told that Atiyoga (excess use) of Kapikachu causes Mada, Vibhrama (Intoxication).

Nighantukaras like Bhavamishra, Kaideva, Sodhala etc in their nighantus mentioned, Rasa, Guna, Virya, Vipaka of Kapikachu in detail along with its dosha karma, rogaghnata and toxic effects. During the nigahntu period, the description of a plant or drug is made through various synonyms. In reviewing literature, 18 synonyms were found for Kapikachu. Among them Atma Gupta, Markati, Kandura, Vrishya, Dusparsha, Shookashimbi are mentioned repeatedly to describe the characters of Kapikachu. The rasa panchaka of this drug as mentioned by most the authors is Madhura, Tikta rasa, Guru guna with Ushna virya, and Madhura vipaka. Dosha karma of this plant is Vatahara. This drug is indicated in various Vatavyadhis, Kapha Pitta Arsha, Atisara. Since this drug is mentioned as effective in causing Brimhana, Balya, Vrishya karma.

At present in the name of Kapikachu 3 varieties are found to be in use, they are

1. *Mucuna pruriens* (L) DC.

2. *Mucuna cochinchinensis* (Lour.) A. Chev. (White seeds),

3. *Mucuna cochichinensis* (Lour.) A. Chev. (Black seeds).

Depending on its availability, locality wise these 3 plants were used as Kapikachu for different purposes. In the market, raw drug dealers are often sold *M. cochinchinensis* in the name of 'Kewatch beej'. Even the villagers and tribal people of many Indian states, are growing this climber in their backyards and use its seeds in the preparation of food (ex. Curry). Hence, it has become necessary to validate the efficacy of these plants by using modern scientific tools and experimental models. So that, the Vrishya activity for which these seeds are being used can be confirmed.

5.2 Pharmacognostical evaluation of *Mucuna* sps.

Pharmacognosy of any plant provides in depth knowledge in terms of its identification, purity and quality of its raw drugs. The authenticity of any drug can be determined with phrmacognostical studies. Here in the current study seeds of Kapikachu (all three samples) were collected from their natural source as well as from local markets, and then authenticated by the experts, and detailed macroscopic and microscopic studies of seeds were carried out. The results of these studies were consistent and identical with the previous studies of *Mucuna* seeds (Yelne and Sharma 1992; Vijayambika *et al.*2011; Kumaresapillai *et al.*, 2013).

5.3 Phytochemical study of *Mucuna* seeds

- Loss on drying of any given sample reflects the moisture content of the drug. More moisture content makes the drug difficult to preserve. In this study loss on drying of all three samples viz *M. pruriens*, *M. cochinchinensis* (White seed), *M. cochinchinensis* (Black seed), found to be 5.56%, 10.15%, 9.29% respectively.

- The ash value indicates the presence of salts and inorganic material in the sample. In the present study ash values of the samples were 3.88%, 3.42%, 3.53% respectively acid insoluble ash 0.09%, 0.04%, .10%; water insoluble ash 0.14%, 0.05%, 0.07% aqueous solution of seeds were alkaline in nature and showed pH 7.59,7.43, 7.40 respectively.

- Extractive values of *M. pruriens*, *M. cochinchinensis* white and black seed samples in different solvents such as, water soluble extractive 29%, 21.89%, 24.82%; Absolute alcohol soluble extractives 9.61%, 13.46%, 14.08%; Petroleum ether soluble extractives 5.34%, 5.17%, 11.41%; Chloroform soluble extractive 8.20%, 8.22%, 15.05%; Carbon tetra chloride soluble extractives 8.20%, 7.61%, 11.12%; Ethyl acetate soluble extractives 5.02%, 5.17%, 9.60%; Acetone soluble extractive 7.41%, 6.07%, 10.74%; methanol soluble extractives 19.05%, 12.32%, 15.83%; Benzene soluble extractive 5.84%, 5.63%, 11.05% and in Hydro-alcoholic soluble extractives 38.64%, 38.13%, 38.05% respectively.

- Qualitative and quantitative analysis of Phytochemical constituents of *M. pruriens*, *M. cochinchinensis* white and black seed samples have shown the presence of different phyto-constituents like alkaloids, saponins, reducing sugars, carbohydrates, proteins, starch, saponins, flavanoides. Steroids were found to be present only in *M. pruriens* seeds.

- Elemental analysis of these three species of *Mucuna* has shown the presence of Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Sulphur, Iron, Manganese, Zinc, Copper, and Sodium in almost equal quantities. No significant differences were observed in the form of quantity among them.

5.4 RAPD based DNA Finger printing of *Mucuna* sps

RAPD analysis in the present study revealed very high level of Polymorphism (92%) for different *Mucuna* species. Padmesh *et al*., (2006) observed that 11 *Mucuna* accessions produced 90.1% polymorphism. Leelambika *et al*., (2010), observed 98% polymorphism in 18 accessions of *Mucuna*. Generated data showed RAPD markers are suitable tools to scan anonymous DNA of species with high secondary metabolite content such as *Mucuna*. The limited reliability of RAPD molecular markers was compensated by increasing the number of RAPD fragments. In this study, the large similarity values revealed by RAPD markers while making the phylogenetic tree provide greater confidence for assessment of genetic relationship among the species. RAPD profiling of these two plants have not yet reported.

5.5 Spermatogenesis

Almost all the species of *Mucuna* sps 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 (Manyam, 1995; Daxenbichler, 1971; British Pharmacopoeia, 1973; Longhi, 2011). L-DOPA plays important role in spermatogensis and recovery of endocrine axis. The effect of L-DOPA were not comparable to *M. Pruriens* however, it suggested that L-DOPA as one of the active principle in *M. 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 (Singh, 2013). The L-DOPA induced facilitation of sexual behavior in the male rats was mediated by dopamine. The effects of L-DOPA were observed in elements preceding and during the copulatory act as described by Malamnas (1976).

L-DOPA possesses antioxidant activity which was supported by *in-vitro* antioxidant assays (Gulcin, 2007). The antioxidant and free radical scavenging activity of Dopamine a metabolic product of L-DOPA was found to be highly significant (Gow-Chin, 1997; Kazuki, 2000). 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.

Shukla *et al*. (2009) had reported that on treatment with *M. pruriens* in infertile men there was significant improvement in serum testosterone, luteinizing hormone, dopamine, adrenaline, and noradrenaline levels and reduced levels of follicle stimulating hormone (FSH) and prolactine hormone (PRL). Sperm count and motility were significantly recovered in infertile men.

M. pruriens seed powder rejuvenates the harmonic balance of male reproductive hormones in infertile men and reactivates the enzymatic activity of metabolic pathways and energy metabolism (Gupta *et al.*, 2011).

5.6 Acute and sub-acute toxicity

The toxicity studies are conducted to know the effect of the drug or chemical on the health of rodent and non-rodent species. These studies will be helpful for the

prediction of harmful or detrimental effects on the human and animals. These studies will also help to know the target organs on which the test substance will act.

The oral route was selected for the study because human beings are more intended to be exposed to the drug through oral route.

The oral sub-acute toxicity was carried out to find out the NOEL of *M. cochinchinensis* (Black seed) as it was found to be more potent than the other *Mucuna* sps *viz., M. pruriens* and *M. cochinchinensis* (White seed) in spermatogenic activity.

The *M. cochinchinensis* (Black seed), *M. pruriens* and *M. cochinchinensis* (White seed), were found to be safe upto 2000 mg/kg body weight when administered orally to rats. The NOEL of *M. cochinchinensis* (Black seed) was found to be 1000 mg/kg body weight when administered orally for 28 days.

Interpretation of results with Ayurvedic back ground is necessary as in Ayurveda it is mentioned by the scholars like Acharya Caraka, Sukra is formed by the best qualities of four mahabhutas except akasa, and by the essence of all six rasas of ahara consumed by every individual (C.S.Sa.2/4). Even though it was told that all six rasas are the reason for forming the Sukra, only madhura rasa helps in forming sukra, all other rasas by their qualities can spoil sukra. Still it was told to have Madhura pradhana shadrasa yukta ahara for the formation of healthy sukra (C.S.Sa.2/4). Both Caraka and Susruta have explained that sukra presents all over the body (C.S.Chi.2, 4/46; S.S.Sa 4/21). The properties of Sukra as mentioned by Acharya Caraka and Susruta (C.S.Chi.2;4/50; S.S.Sa.2/11), in their texts, are exactly opposite to the qualities of Vata, and predominantly matches with Kapha and Madhura rasa pradhana. Hence, the ahara (Food) and vihara (Activities) which increases vata are definite reasons for sukra kshaya. Even Caraka stated in his text C.S.Ni.6/8, vata is the sole reason which causes Sukra kshaya. Drugs like Kapikachu which act as Vata hara are much useful in the conditions where sukra kshaya is present.

The findings from present study suggest that the drug like Kapikachu, by virtue of its Madhura rasa, snigdha, guru guna, madhura vipaka pacifies vata and helps in the formation of Sukra in both healthy individuals and those suffering by vata. It is also observed that the body weight of experimental animals was also increased. It also proves the wisdom of Ayurveda Acharyas of keeping kapikachu in ‘Balya gana’.

Chapter 6: Conclusions and significant findings

As mentioned earlier in the introduction chapter the all aims and objectives of this study were achieved. The achievements of this study can be concluded in short as follows:

1. Pharmacognostic studies in detailed were carried out and identification markers for the three samples of *Mucuna* were established using morphological, macroscopic, microscopic and powder microscopic characters. The differences between 3 *Mucuna* samples at various levels are mentioned, described and highlighted in comparative account section (section 4.4, pp 101) in observation and results chapter. This comparison will be helpful in identification and authentication of the species. During the course of study, it has been observed that there are certain morphological characters that can be observed with naked eye at some stages, with noticeable differences in flower colour, pod colour, presence or absence of hairs etc. Since, these morphological differences might vanish after particular stage / ripening, and *M. pruriens* and *M. cochinchinensis* may look similar. The establishment cum development of identification markers or key characters of these three species has become necessary at different levels of plant development.
2. Physical constant required to develop pharmacopoeial standards associated with qualitative and quantitative phytochemical analysis of the various extracts of seeds revealed the presence of various phyto-constituents *i.e.* carbohydrates, proteins, steroids, saponins, flavonoids, alkaloids, tannins and phenols. In this study, all the tests were carried out as per internationally accepted standard guidelines, as laid down by WHO and API. Initially Parameters like foreign matter, total ash, acid insoluble ash, water soluble ash, loss on drying, pH, swelling factor etc were evaluated to find out the differences among these three species as they all look similar in powder form. After this initial study all these three seeds in powder form were subjected to different extractive procedures in various solvents including absolute alcohol, water, methanol, acetone, ethyl acetate, petroleum ether, benzene, chloroform, carbon tetra chloride, hydro-alcoholic (1:1). By experimenting it is understood that, the extractive values of each species in respective solvent, happens to be different from others and this also can be used as

a valid and scientific tool to identify and differentiate the seed powders of all three species from each other. Apart from these, extractive values of the seed powders of *Mucuna* sps, presence of other phyto constituents and their quantified values for Carbohydrates, Flavanoids, Tannins, Glycosoides, Proteins, Total Phenolic contents, Lipids were also analyzed and documented in detailed. Along with this microelements *viz.*, Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Sulphur, Iron, Manganese, Zinc, Copper, Sodium has thrown a light to better understand the results of these plants shown on *Vrishya* (spermatogenesis) activity which is the main indication of this drug. Vast differences in quantity wise were found in respect of carbohydrates, proteins, lipids, glycosoides and tannins among all these three species.

In this study, the quantification of L-Dopa, the main ingredient of this species, was also done, to highlight the detection of L-Dopa species and quantity wise in the seed powder of *Mucuna* sps. The knowledge of L-Dopa quantity present in different species helps in using this seed powder for different purposes *viz*. use in Parkinson's disease, purpose of spermatogenesis. In present study, huge differences among the quantity were found among these three species and specifically *M. cochinchinensis* black seed was found to be having maximum quantity of L-Dopa.

3. DNA fingerprinting with RAPD-PCR (Random Amplification of Polymorphic DNA-Polymerase chain reaction) technique of three samples of *Mucuna* was carried out, to establish an identification key at molecular level, among all these three *Mucuna* sps. This helped in distinguishing the plants, at juvenile stage of the plant as well as even with the traceable quantity. This technique is more reliable, accurate and universally accepted by world scientific community. In this study, all three species have shown different successive polymorphic banding patterns with primers which indicate the presence of significant genetic variations among the species.
4. The antioxidant activity assay of the aqueous extracts of all three *Mucuna* sps, was performed to validate the classical uses of Kapikachu, Vaghbhata mentioned for its Rasayana activity. Moreover, our findings have shown the presence of different constituents, which are responsible for antioxidant activities. Hence the presence of these constituents, reflected potent antioxidant activity for reducing the possible

damage of cellular constituents, due to oxidative stress and in response protecting the tissues from various degenerative disorders.

5. Acute oral toxicity studies on *Mucuna pruriens, Mucuna cochinchinensis* (Black seed) and *Mucuna cochinchinensis* (White seed) were carried out using Sprague dawley rats, to evaluate the toxic effects of seed powder. This study has proved the safety of seed powder of all three species, even at the dose of 2000 mg/kg body weight (as single dose), which has not exhibited any toxic effect till 14 days of experimental observations.
6. The *Mucuna cochinchinensis* (Black seed) has been found to have more potent spermatogenetic activity than other varieties of Kapikachu *i.e.*, *Mucuna cochichinensis* (White seed) and *Mucuna pruriens* in rats. The present study revealed the potency of spermatogenesis activity at different doses *viz.* 100 mg/kg, 200 mg/kg, 400 mg/kg body weight for 30 days. It has also been observed that at the dose of 400 mg/kg body weight seed powder of *Mucuna cochinchinensis* (Black seed) and seed powder of *Mucuna pruriens*, and standard drug at the dose of 20 mg/kg body weight has shown significant increase in sperm count, motility and morphology ($p<0.05$). Statistical analysis of this experimental study were carried out and found to be significant at $p<0.05$.
7. The no observed adverse effect level (NOAEL) of *Mucuna cochinchinesis* (Black seed) in rats, following Oral administration for 28 days was found to be more than 1000 mg/kg body weight. At the end of this sub-acute study no noticeable abnormality was found, similarly treatment related mortality in both male and female rats up to the dose of 100 mg/kg body weight was not observed. Finally no significant gain in body weight was reported (in male and female rats up to the dose of 100 mg/kg body weight).

Chapter 7: Bibliography

Ahmad MK, Mahdi AA, Shukla KK, Islam N, Jaiswar SP, Ahmad S (2008), Effect of *Mucuna pruriens* on semen profile and biochemical parameters in seminal plasma of infertile men. *Fertil Steril*, **90**:3.

Aiyer NK, Kolamma M (1962), Pharmacognosy of Ayurvedic Drugs, Kerala, Dept. of Pharmacognosy, University of Kerala, Trivendrum, **1(5)**: 47-150.

Albert MJ, Bhuiyan NA, Talukder KA, Faruque ASG, Nahar S, Faruque SM, Ansaruzzaman M, Rahman M (1997), Phenotypic and genotypic changes in *Vibrio cholerae* 0139 Bengal. *J. Clini. Microbio*, **35**: 2588–2592.

Amin KMY, Khan NA, Rahman SZ (1993), The Sezual Function improving effect of Tukhm-e-Kounch (*Mucuna pruriens*) and its mechanism of action- An experimental study, *Proc. 1st National Seminar on II mul Advia, Beenapura*, 25th April, Abst.No.48

Amin KMY, Khan MN, Zilur-Reheman S, Khan NA, (1996), Sexual function improving effect of *Mucuna pruriens* in sexually normal rates. *Fitoterapia*, **67(1)**:53-58.

Anantha Kumar KV, Srinivasan KK, Shanbhag T, Rao SG, (1994), Aphrodisiac Activity of the seeds of *Mucuna pruriens. Indian Drugs*, **31(7):** 321-327.

Anonymous (1962), The Wealth of India, Raw Materials, CSIR, Publication and Information Directorate, New Delhi, Vol. 6 (L-M), PP. 442.

Anonymous (1970), Charaka Samhita, Commentry by Shastri, K.N. Chaukhambha Vidyabhavan, Varanasi.

Anonymous (1980), Sushruta Samhita, ed. Acharya, J.T., Chaukhambha Orientalia, Varanasi.

Anonymous (1982), Ashtanga Hridayam, ed. Kunte, A.M. Chaukhambha Orientalia, Varasasi.

Anonymous (1987), Medicinal Plants of India, Indian Council of Medical Research, New Delhi, Vol.2, PP.282-287.

Anonymous (1990), Phytochemical Investigation of Certain Medicinal Plants used in Ayurveda, CCRAS, New Delhi. PP. 41.

Anonymous (1995), Indian Medicinal Plants, Varier's, P.S. Arya Vaidya shala , Kottakkal, Orient Longman Ltd., Vol. 4, PP, 68-72.

Anonymous (2001), The Ayurvedic Pharmacopoeia of India, Ministry of Health & Family Welfare. Department of Indian Systems of Medicine & Homeopathy. Part I vol. III 1st edn. : 23-24.

Anonymous (2003), Quality Standards of Indian Medicinal Plants, Indian Council of Medical Research (ICMR), New Delhi, Vol. I, PP. 136-141.

Anonymous. (1998), Quality Control Methods for Medicinal Plants Materials. Geneva: World Health Organization : 6-22.

Baker HG, Stebbins GL (1965), The Genetics of Colonizing Species. Academic Press, New York, New York, USA.

Barrett SH, (2015), Foundations of invasion genetics: the Baker and Stebbins legacy. *Molecular Ecology*, **24**: 1927-1941.

Bedi SJ, (1978), Ethnobotany of the Ratanmahal Hills, Gujarat, India, *Etno. Bot.*, Vol. **32(3):** 278-284.

Bell EA, Janzen DH (1971), Medical and Ecological consideration of L-DOPA and S-HTP in seeds, *Nature*, **229:**136.

Beta T, Nam S, Dexter JE, Sapirstein HD (2005), Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. *Cereal Chem*. **82**:390–393.

Bhaskar A, Nithya V, Vidhya VG. (2011), Phytochemical evaluation by GC-MS and antihyperglycemic activity of Mucuna pruriens on Streptozotocin induced diabetes in rats. *Journal of Chemical and Pharmaceutical Research*, **3(5):** 689-696.

Bhaskar A, Vidhya VG, Ramya M. (2008), Hypoglycemic effect of Mucuna pruriens seed extract on normal and streptozotocin-diabetic rats. *Fitoterapia.* **79(7-8)**:539-543.

Bhat KV, Babrekar PP, Lakhanpaul S. (1999), Study of genetic diversity in Indian and exotic sesame (*Sesamum indicum* L.) germplasm using random amplified polymorphic DNA (RAPD) markers. *Euphytica*, **110**: 21-34.

Birajdar VV, Mhase AG, Gurav AM, Murthy SN. (2014), Preliminary pharmacognostic and phytochemical standardization of Dhataki [*Woodfordia fruticosa* (L.) Kurz.] leaves. *Ayu*., **35**: 309-15.

Blois, MS. (1958), Antioxidant determinations by the use of a stable free radical. *Nature*. **181**: 1199-1200.

Brain KR. (1976), Accumulation of L-DOPA in Cultures from *Mucuna Pruriens Plant Sci. Lett.*, **7(3)**: 157-161.

Bravo L, Siddhuraju P, Saura-Calixto F. (1998), Effect of various processing methods on the in vitrostarch digestibility and resistant starch content of Indian pulses. *J. Agric. Food. Chem.* **46**: 4667–4674.

Capo-chichi LJA, Weaver DB, Morton CM. (2003), The use of molecular markers to study genetic diversity in *Mucuna*. *Tropical and Subtropical Agroecosystems*, **1:** 309-318.

Cargile, N, Dorenbos, NJ (1976), New analytical procedures for L-DOPA in plant materials, *J. Miss. Acad. Sci.*, Vol. **21**: 50-53.

Carlos FV, Victor P, Peter F, Jorge L. (2006), Genetic diversity and structure in fragmented populations of the tropical Orchid *Myrmecophila christinae* var *christinae*. *Biotropica*, **38:**754 –763.

Chase CR, Pratt RJ. (1949), Fluorescence of powdered vegetables drugs. *Indian Journal of Experimental Biology*, **33(6)**: 428-432.

Chatterjee A, Prakashi SC. (1992), The Treatise on Indian Medicinal Plants, Publications & Information Directorate, New Delhi, Vol. 2, PP. 102-103.

Chauhan MG, Pillai APG. (2011), Microscopic Profile of Drugs Used in Indian Systems of Medicine, Gujrat Ayurved University, Jamnagar, Vol.3, Seed Drugs, Part I: 107-108.

Chopra RN, Nayar SL, Chopra IC. (1956), Glossary of Indian Medicinal Plants, CSIR, New Delhi, PP. 171.

Chunekar KC. (1982), "Bhavprakasa Nighantu", Indian Materia Medica of Sri. Bhavamisra, Chaukhambha Bharati Academy, Varanasi, PP. 357.

Chunekar KC. (2013), Bhavprakasa Nighantu, Indian Materia Medica of Sri. Bhavamisra, Chaukhambha Bharati Academy, Varanasi, PP. 342-343.

Cook T. (1967) The Flora of the Presidency of Bombay. Botanical Survey of India, Calcutta. 2nd reprint Vol. I: 389-390.

Davide Grassi, Giovambattista Desideri; Claudio Ferri. (2010), Flavonoids: Antioxidants against atherosclerosis. *Nutrients* **2**: 889-902.

Daxenbichler ME, Van Etten CH, Hallman EA, Earle FR, Barclay AS. (1971), Seeds as sources of L-DOPA. *J. Med. Chem.* **14**: 463.

Desai SM, Sharon M, Sharan M. (2010), Effect of Culture Conditions on L-Dopa Accumulation in Callus Culture of *Mucuna pruriens*. *J. Chem. Pharm. Res*, **2(4)**, 134-146.

Dhanasekar P, Dhumal KN, Reddy KS. (2010), Identification of RAPD markers linked to plant type gene in pigeonpea. *Indian J. Biotech*, **9:** 58 – 63.

Dhawan SS, Rai GK, Darokar MP, Lal Rk, Misra HO, Khanuja SPS. (2011), Comparative genetic analysis of thrichome-less and normal pod genotypes of *Mucuna pruriens* (Fabaceae). *Genetics and molecular research*, **10 (3)**: 2049-2056.

Dhyani SC. (1994), Rasa-Panchaka (Ayurvedic Principles of Drug Action) Krishnadas Academy, Varanasi.

Dvivedi Laxshmidhar (2013), Caraka Samhita of Maharshi Agnivesa editor and Commentator by Dr. Laxshmidhar Dvivedi, Chowkhambha Krishnadas Academy, Varanasi.

Ekanem AP, Obiekezie A, Kloas W, Knopf K. (2004), Effects of crude extracts of *Mucuna pruriens* (Fabaceae) and *Carica papaya* (Caricaceae) against the protozoan fish parasite *Ichthyophthirius multifiliis*. *Parasitol Res*, **92**:361–366.

Eze JI, Ndukwe S. (2012), Effect of methanolic extract of *Mucuna pruriens* seed on the immune response of mice. *Comp Clin Pathol*, **21**:1343–1347.

Fung SY, Tan NH, Liew SH, Sim SM, Aguiyi JC. (2009), The protective effects of *Mucuna pruriens* seed extract against histopathological changes induced by Malayan cobra (*Naja sputatrix*) venom in rats. *Trop Biomed*, **26(1)**:80-84.

Fung SY, Tan NH, Sim SM, Marinello E, Guerranti, Aguiyi JC. (2011), *Mucuna pruriens* Linn. Seed extract pretreatment protects against cardiorespiratory and neuromuscular depressant effects of *Naja sputatrix* (Javan spitting cobra) venom in rats. *Indian Journal of Experimental Biology* **49:**254-259.

Fung SY, Tan NH. (2012), Effect of *Mucuna pruriens* seed extract pretreatment on the responses of spontaneously beating rat Atria and Aortic Ring to *Naja sputatrix* (Javan Spitting Cobra) Venom. *Journal Evidence-Based Complementary and Alternative Medicine*, Article ID 486390 (http://dx.doi.org/10.1155/2012/).

Gad SC. (1982), A neuromuscular screen for use in industrial toxicology, *J. Toxicol. Environ. Health*; **9:** 691-704.

Ganai BA, Masood A, Zargar MA. (2005), Experimental Biochemistry, 1st ed., Valley Publications, J&K: 148-149.

Garg Sunita (1992), Substitute and Adulterant Plant, Periodical Expert Book Agency, New Delhi, pp.82.

Ghosh G. (1983), Pharmacognostic Study of *Mucuna pruriens* Baker Non D.C. seed, *Nagarjuna*, Vol. **27**: 51-64.

Girijadayalu Suklah (2006), “Bhelasamhita”, Chaukhambha Bharati Academy, Varanasi.

Gogate VM. (2000), Ayurvedic Pharmacology and Therapeutic Uses of Medicinal Plants (Dravyagunavidnyan), Bharatiy Vidhyabhavan (SPARC), Mumbai, PP. 329-331.

Gosh G. (1982), Note on pharmacognostic and chemical identification of *Mucuna utlis* seeds, a substitute of *Mucuna pruriens*, *Indian Drug*, **20(1)** : 24-25.

Gow-Chin Y, Chiu-Luan H. (1997), Antioxidant effects of dopamine and related compounds. *Biosci Biotech Biochem*, **61(10):** 1646–1649.

Graham HD. (1992), Stabilization of the Prussian blue color in the determination of polyphenols. *J. Agric. Food Chem.* **40**: 801–805.

Gulcin I. (2007), Comparison of *in vitro* antioxidant and antiradical activities of L-tyrosine and L-Dopa. Amino Acids, **32:** 431–438.

Gupta A, Mahdi AA, Ahmad MK, Shukla KK, Bansal N, Jaiswer SP, Shankhwar SN. (2011), A proton NMR study of the effect of *Mucuna pruriens* on seminal plasma metabolites of infertile males. *J Pharm Biomed Anal*, **55:**1060–1066.

Harborne JB. (1998), Phytochemical Methods - A Guide to Modern Techniques of Plant Analysis. 1st edn: Chapman and Hall; London, UK.

Harbourne JB. (1984), Phytochemical Methods: A Guide to Modern Technique of Plant Analysis, 2nd ed. Chapman and Hall, London.

Hishikar R, Shastry S, Shinde S, Gupta SS. (1981), Preliminary Phytochemical and anti-inflammatory activity of seeds of *Mucuna pruriens*, *Indian J. Pharmacol.*, **13(1)**: 97-98.

http://www.tropicalforages.info/key/Forages/Media/Html/Mucuna_pruriens.htm

http://saffron.pharmabiz.com/article/detnews.asp?articleid=16449&sectionid=50

http://infertility.health-info.org/male-infertility/male-infertility-sperm-disorders.html

Huizing HJ, Wijnsma R, Batterman S, Malingre TM, Wichers HJ. (1985), Production of L-DOPA by cell suspension cultures of *Mucuna pruriens*; (I. Initiation and maintenance of cell suspension cultures of *Mucuna pruriens* and identification of L-DOPA. *Plant Cell Tissue Organ Culture*, **4**: 61-73.

Hussain G, Manyam BV. (1997), *Mucuna pruriens* proves more effective than L-DOPA. In Parkinson's disease animal model, *Phytotherapy Research*, **11(6):** 419-423.

Indradev Tripathi (2010), "Raja Nighantu" of Pandit Narhari, Chowkhambha Krishnadas Academy, Varanasi, PP-38.

Infante ME, Perez AM, Simao MR, Manda F, Baquete EF, Fernandes AM. (1990), Outbreak of acute toxic psychosis attributed to *Mucuna pruriens*. The Lance, **336**:1129.

Jadhav SR, Dadhich OP, Kothari P. (2015), KAPIKACCHU (*Mucuna pruriens*) - A Ayurvedic drug review, *World J Pharm Sci* . **3(10)**:1999-2003.

Jadhav SR. (2013), Physiological study of Shukravaha Srotas and clinical study of kapikacchu Churna in Klaibya with special Ref. to oligozoospermia" (Thesis), PG Dept. of Sharir kriya, NIA Jaipur :141-145.

Jadhavji Trikamji Acharya (2010), "Caraka Samhita" by Agnivesa, Chaukhambha Krishnadas Academy, Varanasi.

Janardhanan K, Gurumoorthi P, Pugalenthi M. (2003) Nutritional potential of five accessions of a South Indian tribal pulse, *Mucuna pruriens* var. *utilis*. Part I. The effect of processing methods on the contents of L-Dopa phytic acid, and oligosaccharides. *Journal of Tropical and Subtropical Agro-ecosystems*, **1:** 141–152.

Johnson DA. (1940), Plant Micro Techniques. New York, London: McGrow Hill Book Company; pp. 105.

Joy Ganem Longhi, Elisa Perez, Jair Jose de Lima, Lys Mary Bileski Candido. (2011), *In vitro* evaluation of *Mucuna pruriens* (L.) DC. antioxidant activity. *Brazilian Journal of Pharmaceutical Sciences*, **47(3)**: 535-544.

Kalidass C, Mohan VR. (2010) Genetic diversity of Velvet bean, *Mucuna pruriens* (L.) DC. from Southern and Western Ghats, India. *International Journal of Biological Technology*, **1(2)**: 31-37.

Kamat SD. (2011), Studies on Medicinal Plants and Drugs in Dhanvantari Nighantu, Vol-1, Chowkhambha Sanskrit Pratishthan, Delhi, PP-55.

Kapoor LD. (2001), CRC Handbook of Ayurvedic Medicinal Plants, Published by CRC press, USA. Pp 1-391.

Kar A, Choudhary BK, Bandyopadhyay NG. (2003), Comparative evaluation of hypoglycemic activity of some Indian medicinal plants in alloxan diabetic rats. *J. Ethnopharmacol*; **84**:105-108.

Katzenschlager R, Evans A, Manson A. (2004), *Mucuna pruriens* in Parkinson's disease: a double blind clinical and pharmacological study. *Journal of Neurology*, Neurosurgery and Psychiatry, **75**:1672–1677.

Kaviraj Ambikadutta Shastri (2012), "Susrutasamhita" of Maharshi Susruta Part 1 & 2, Chaukhambha Sanskrit Sansthan, Varanasi.

Kavitha C, Thangamani C. (2014), Amazing bean "*Mucuna pruriens*": A comprehensive review. *Journal of Medicinal Plants Research*, **8(2)**:138-143.

Kazuki K, Hiroyuki S. (2000), High content of dopamine, a strong antioxidant, in Cavendish banana. *J Agric Food Chem*, **48:** 844–848.

Khandelwal KR. (2001), Practical Pharmacognosy Technique and Experimental. Nirali Prakashan Pune, : 149-156.

Khare CP. (2004), Encyclopedia of Indian medicinal plants. Springerverley Berlin Heidelberg.

Khatiwora E, Adsul VB, Kulkarni MK, Deshpande NR, Kashalkar RV. (2010), Spectroscopic determination of total phenol and flavonoid contents of *Ipomoea carnea*. *International Journal of Chem Tech Research*, **2(3)**: 1698-1701.

Kirtikar KR, Basu BP. (1933), Indian Medicinal Plants, L.M. Basu, Allahabad, Vol.1, PP.778-780.

Kokate CK. (1996), Practical Pharmacognosy. 4th ed. Reprint. Vallabh Prakashan Delhi : 10-321.

Krishnaveni S, Theymoli B and Sadasivam S. (1984), Phenol Sulphuric acid method. *Food Chem*. **15**: 229.

Kulkarni Yogini, Sane Hema (2014), Botany of Commonly used Medicinal Plants with Diagnostic keys, Vision Publication, Pune. PP. 110.

Kumar A, Rajput G, Dhatwalia VK, Srivastav G. (2009), Phytocontent Screening of *Mucuna* Seeds and Exploit in Opposition to Pathogenic Microbes. *Journal of Biological0 and Environmental Sciences*, **3(9):**71-76.

Kumar DS, Muthu AK. (2010), Free radical scavenging activity of various extracts of whole plant of *Mucuna pruriens* (Linn): An *in-vitro* evaluation. *Journal of Pharmacy Research*, **3(4):**718-721.

Kumar P, Saha S, (2013), An updated review on Taxonomy, Phytochemistry, Pharmacology and Toxicology of *Mucuna pruriens*. *Journal of Pharmacognosy and Phytochemistry*, **2(1):**306-14.

Kumaresapillai Natarajan, Nallaperumal Narayanan, Natesan Ravichandran (2013), Pharmacognostical characterization, phytochemical and antimicrobial evaluation of Mucuna cochinchinensis (lour.) Cheval seeds. *Journal of pharmacy research*, **6:**318-323.

Laurena A.C, Revilleza MJR, Mendoza EMT. (1994) Polyphenols phytate, cyanogenic glycosides and trypsin inhibitor activity of several Philippine indigenous food legumes. *J. of Food Comp. and Analys*, 7: 194–202.

Leelambika M, Mahesh S, Jaheer M, Sathyanarayana N. (2010), Comparative evaluation of genetic diversity among Indian *Mucuna* species using morphometric, biochemical and molecular approaches. *World Journal of Agricultural Sciences,* **6(5):** 568-578.

Lim A, Yue GH, Lim-Ho CL, Hong Y. (2007), Development and characterization of microsatellites in *Vanda* varieties. *Mol Ecol Notes,* 7**:** 461 – 463.

Longhi JG, Perez E, Jose de Lima J, Candido LMB. (2011) *In vitro* evaluation of *Mucuna pruriens* (L.) DC. antioxidant activity. *Brazilian Journal of Pharmaceutical Sciences*, **47(3):** 535-544.

Lorenzetti E, MacIsaac S, Arnason JT, Awang DVC, Buckles D. (1998), The phytochemistry, toxicology and food potential of velvet bean (Mucuna Adans spp., Fabaceae) Cover crops of West Africa: contributing to sustainable agriculture. IDRC, Ottawa, Canada and IITA, Ibadan, Nigeria. 1998; 57.

Lorenzetti F, MacIsaac S, Arnason JT, Awang DVC, Buckles D. (2015), The phytochemistry, toxicology, and food potential of velvetbean (*Mucuna* Adans. spp., Fabaceae) http://www.idrc.ca/en/ev-31916-201-1-DO_TOPIC.html, last visitied on 24th October 2015.

Lowry OH, Rosebrough NR, Lewis Farr A, Randall RJ. (1951), Protein measurement with the Folin Phenol Reagent. *J. Biol. Chem*, **193**: 265-275.

Lucas DS. (2013), Dravyagun Vijnana study of Dravya-Materia Medica, Choukhambha Bharati Academy, Vol.2, PP. 123-126.

Madham Shetty, Suresh Babu (2012), “CAKRADATTA”, Chowkhambha Krishnadas Academy Varanasi.

Madham Shetty, Suresh Babu (2011), “Yoga Ratnakar”, Vol-1 and 2, Chowkhambha Sanskrit Series Varanasi.

Mallick CP, Singh MB. (1980), Plant enzymology and Histoenzymology. Kalyani publishers, New Delhi, 286.

Malmnas CO. (1976), The significance of dopamine, versus other catecholamines, for L-dopa induced facilitation of sexual behavior in the castrated male rat. *Pharmacol. Biochem. Behav.*, **4(5):** 521-526.

Manjunatha BK, Patil HSR, Vidya SM, Kekuda TRP, Mukunda S, Divakar R. (2006), Studies on the antibacterial activity of *Mucuna monosperma* DC. *Indian Drugs* **43**:150-152.

Manueli AM, Jerge L, Karl-Josef Dietz, Pedro M, Aparicio T, Maniust Becana (2010), Functions of antioxidant enzymes and metabolites during maturation of Pea fruits. *Journal of Experimental Botany*, **6(1)**: 87-97.

Manyam BV, Dhanasekaran M, Theodore AH, Neuroprotective, (2004), Effects of the Antiparkinson Drug *Mucuna pruriens. Phytotherapy Research,* **18**:706–712.

Manyam BV. (1995) An alternative medicine treatment for Parkinson's disease: results of a multicenter clinical trial – HP-200 in Parkinson's disease study group. *J. Alternat. Comp. Med.*, **1**: 249-255.

Meenatchisundaram S, Michael A. (2010), Antitoxin activity of *Mucuna pruriens* aqueous extracts against Cobra and Krait venom by *in vivo* and *in vitro* methods. *Int. J. Pharm. Technol Res.*, **2(1)**: 870-874.

Mehta JC, Majumdar DN. (1994), Indian medicinal plants-V *Mucuna pruriens* bark (N.O; Papilionaceae). *Ind J Pharm.*, **6**: 92–94.

Mishra L, Wagner H. (2004), Alkaloidal constituents of Mucuna pruriens seeds. *Phytochemistry*, **65:** 2565–2567.

Mishra L, Wagner H. (2006), Lipid derivatives from Mucuna pruriens seeds. *Indian Journal of Chemistry*, **45(B)**: 801-804.

Mittler R, Vaderauwera S, Gollery M, Van Breusegen F (2004), Reactive oxygen gene network of plants. *Trends in Plant Science,* **9:** 490-498.

Mohan VR, Janardhanan K. (1995), Chemical analysis and nutritional assessment of lesser-known pulses of the genus *Mucuna. Food Chemistry,* **52**: 275–280.

Mosa Q. PHG 322 Practical Course: lab # 2 Quantitative analysis of Glycosides. Pharmacognosy Department, King Saud University, 1-10.

Moser VC, McDaniel KL, Phillis PM. (1991), Rat strain and stock comparisons using functional observational battery: baseline values and effects of Amitraz. *Toxicol. Apl. Pharmacol*., **108**: 267-283.

Mugendi JB, Njagi EM. (2010), Effects of processing technique on the nutritional composition and anti–nutrient content of *Mucuna* bean *(Mucuna pruriens L.). African Journal of Food Science*, **4(4):** 156-166.

Nadkarni KM (1976), Indian Materia Medica, Bombay Popular Prakashan, Mumbai, pp. 818-820.

Nagashayana N, Sankarankutty P, Nampoothiri MR, Mohan PK, Mohan KKP. (2000), Association of L-DOPA with recovery following Ayurveda medication in parkinson's disease. *J. Neurol. Sci.,* **176(2)**:124-127.

Naik VN (1998), Flora of Marathwada. Amrut Prakashan, Aurangabad, Vol 1, PP. 290.

Narayan Das (1952), "Rajvallabha Nighantu" Dravya Chandrika Tikasahit, Gangavishnu Krishnadas 'Laxmivyankateshwar' Steampress, Mumbai.

Natarajan K, Nallaperumal Narayanan, Natesan Ravichandran (2013), Pharmacognostical characterization, phytochemical and antimicrobial evaluation of *Mucuna cochinchinensis* (Lour.) Cheval seeds. *Journal of Pharmacy Research*, **6**: 318-323.

Natarajan K, Narayanan N, Ravichandran N. (2012), Review on "mucuna" - the wonder plant. *Int. J. Pharm. Sci. Rev. Res.*, **17(1)**: 86-93.

Nishteswar K, Hemadri K. (2013), Dravyaguna Vijnana, Detailed Study of the Drugs, Choukhambha Sanskrit Pratishthan, Delhi, Vol.2, PP. 43-46.

Nishteswar K. (2008), Basic Concepts of Ayurvedic Pharmacology, Chowkhambha Sanskrit Series office, Varanasi.

Nishteswar K, Vidyanath R. (2014), “Sahastrayogam”, Chowkhambha Sanskrit Series office, Varanasi.

Ovallath S, Deepa P. (2013), The history of parkinsonism: descriptions in ancient Indian medical literature. Mov Disord, **28(5)**: 566-568.

Padmesh P, Reji JV, Dhar M, Seeni S. (2006), Estimation of genetic diversity in varieties of *Mucuna pruriens* using RAPD. *Biologia plantarum*, **50:** 367-372.

Pandey G. (2001), *Dravya guna vijnana*, Krishnadas Academy, Varanasi, Vol.2, PP. 108-115.

Pandey Gyanedra (2014), “Harit Samhita”, Vol 1 and 2, Chowkhambha Sanskrit Series office, Varanasi.

Pandey Gyanendra (2005), “Bhaisajya Ratnavali”, Chowkhambha Sanskrit Series office, Varanasi.

Pandey Gyanendra (2009), “Sodhala Nighantuh”, Chowkhambha Krishnadas Academy, Varanasi, PP. 40.

Pandit Hariprasad Tripathy (2008), “Dhanvantari Nighantu”, Chowkhambha krishnadas Academy, Varanasi, PP. 42.

Pandit Hariprasad Tripathy (2009), “Harita Samhita”, Chowkhambha Krishnadas Academy, Varanasi.

Pandit Hariprasad Tripathy (2009), “Vangasen Samhita”, Chowkhambha Sanskrit Series office, Varanasi.

Pant MC, Uddin I, Bhardwaj UR and Tewari RD (1968). Blood sugar and total cholesterol lowering effect of *Glycine soja*, *Mucuna pruriens* and *Dolichos biflorus* seed diets in normal fasting albino rats. *Ind. J. Med. Res.*, **56**: 1808-1812.

Parab GV, Krishnan S, Janarthanan MK, Sivaprakash KR, Parida, A. (2008). ISSR and RAPD markers assessed genetic variation of *Aerides maculosum* – an epiphytic orchid from Goa, India. *J Plant Biochem Biotech*, **17**: 107-109.

Paranjape Prakash (2012), Indian Medicinal Plants forgotten Healers, Chowkhambha Sanskrit Pratishthan, Delhi.

Parikh KM, Doshi VJ, Sawant SV, Salunkh, UB (1990), Estimation of L-DOPA from the plant *Mucuna pruriens* and its formulations. *Indian Drugs*, **27(6):** 353-356.

Patil D, Pandey DK, Mahesh R, Kurdekar V, Jhadav HR, (2010), Anti-Depressant-like activity of *Mucuna Pruriens*; A Traditional Indian Herb in Rodent Models of Depression. *Pharmacology online,* **(1)**: 537-551.

Pawel S, Jerzy AP, Dariusz Z. (2009), RAPD Markers Reveal Genetic Diversity in *Salix purpurea* L. *Crop Sci.,* **49**: 857-863.

Pradeep Kumar, Sudipta Saha (2013), An updated review on Taxonomy, Phytochemistry, Pharmacology and Toxicology of *Mucuna pruriens. Journal of Pharmacognosy and Phytochemistry*, **2(1):** 306-314.

Prajapati ND, Purahit SS, Sharma AK, Kumar T. (2003), A Handbook of Medicinal Plants, Agrobios, India.

Puri HS (1972), Aphrodisiacs in India- An annotated bibliography of Indian medicine. *Indian Drugs,* **9(9):** 11-14.

Rafalski A, Tingey S, Williams JGK (1993), Random Amplified Polymorphic DNA (RAPD) markers. In: Gelvin SB, Schilperoort RMS, Verma DPS eds. Plant molecular biology manual suppl 6 Dordrecht: Kluwer Academic Publishers, pp. 1-9.

Rajaram N, Janardhanan K. (1991), The biochemical composition and nutritional potential of the tribal pulse, *Mucuna gigantea* (Willd.) DC. *Plant Foods for Human Nutrition,* **44**: 45-51.

Rajeshwar Y, Gupta M, Mazumder UK. (2005), Antitumor Activity and in vivo Antioxidant Status of Mucuna pruriens (Fabaceae) Seeds against Ehrlich Ascites Carcinoma in Swiss Albino Mice. *Iranian Journal of Pharmacology and Therapeutics*; **4(1):** 46-53.

Ramaswamy S, Nazimudeen SK, Viswanathan S, Kulanthivel P, Rajeskaran V. Kameswaran L. (1979). Some Pharmacological effects of *Mucuna pruriens, Proc. M.M.C Res. Soc.*, 2: 39.

Ranade SA, Rana TS, Srivastava AP, Nair KN (2006), Molecular differentiation in *Murraya* Koenig ex L. species in India inferred through ITS, RAPD and DAMD analysis. *Current Science*, **90:** 1253 – 1258.

Rastogi RP, Mehrotra BN. (1993), Compendium of Indian Medicinal Plants, Central Drug Research Institute, Lucknow and Publications and Information Directorate, New Delhi, Vol. 1, PP. 281.

Rastogi RP, Mehrotra BN. (1993), Compendium of Indian Medicinal Plants, Central Drug Research Institute, Lucknow and Publications and Information Directorate, New Delhi, Vol. 2, PP. 472.

Rathi SS, Grover JK, Vats V (2002), The effect of *Momordica charantia* and *Mucuna pruriens* in experimental diabetes and their effect on key metabolic enzymes involved in carbohydrate metabolism. *Phytotherapy Research,* **16(3):** 236–243.

Rayavarapu AK, Kaladhar DSVGK. (2011), Evaluation of antimicrobial activity of *Mucuna pruriens* on plant pathogens. *Asian J. Biochem. Pharmaceut. Res,* **2(1)**: 593-600.

Reynolds JEF. (1989), Martindale: the extra pharmacopoeia. The Pharmaceutical Press, London, UK. pp. 1015–1020.

Rieseberg LH. (1996), Homology among RAPD fragments in interspecific comparisons. *Mol. Ecol.*, **5**: 99-105.

Sadasivam S, Manickam A. (1996), Biochemical methods. New age International (P) Limited, Publishers, New Delhi.

Saini M, Singh S, Hussain Z, Yadav A. (2010), RAPD analysis in mungbean (*Vigna radiata* (L.) Wilczek): I. Assessment of genetic diversity. *Indian J. Biotech.*, **9**: 137-146.

Saksena S, Dixit VK. (1987), Role of the total alkaloids of *Mucuna Pruriens* Baker in spermatogenesis in albino rats. *Indian J. Natl. Prod.*, **3(1)**: 3.

Saligram Vaishya (2011), "Saligramnighantu Bhushanam (Brihatnighantu Ratnakarantargat 7-8 part), Mumbai; Khemraj ShriKrishnadass : pp. 258-259

Sambasivarao K, Tripathi HC, Jawahar Lal, Gupta PK. (1982), Influence of drugs on male sex behaviour and its pharmacological aspects- A mini review, *Indian Drugs*, **19(4):** 129-133.

Sastry JLN. (1954), "Madanpal Nighantu" by Nrupamadanpal, Laxmivyankateshwar Steam Press, Kalyan, Bombay, Abhayadivarga Shlok.70-71, PP. 225-226.

Sastry JLN. (2014), *Dravya guna vijnana*, Chowkhambha Orientalia, Varanasi, Vol. 1, PP.175-179.

Sastry JLN (2010) Illustrated Madanpala Nighantu, Chowkhambha Orientalia, Varanasi.

Sathyanarayana N, Vikas PB, Bharath Kumar TN, Rajesha R. (2010), RAPD markers for genetic characterization in *Mucuna* species. *Indian J Genet.*, **70(3)**: 296-298.

Sekar S, Elumalai P, Seppan P. (2009), Dose- and time-dependent effects of ethanolic extract of *Mucuna pruriens* Linn. Seed on sexual behaviour of normal male rats. *J Ethnopharmacol*, **122:** 497-501.

Sharma PC, Yelne MB, Dennis TJ. (2000), Database on Medicinal Plants used in Ayurveda, CCRAS, New Delhi, Vol. 1, PP. 210-215.

Sharma PV. (1979), "Kaiyadev Nighantu", translated by Guruprasad Sharma Chaukhambha Orientalia, Varanasi, Oushadhi varga, PP. 111-154.

Sharma PV. (1982), "Dhanvantari Nighantu", translated by Guruprasad Sharma Chaukhambha Orientalia, Varanasi, 46/151-154.

Sharma PV. (1972), Indian Medicine in the Classical age, Chowkhambha Amarabharati Prakashan, Varanasi.

Sharma PV. (1994), “Cakradatta”, Chaukhambha Oriental, Varanasi, Vrushyadhikara, 66/6, 9; 26-34.

Sharma PV. (1995), *Dravyaguna vijnana*, Chowkhambha Orientalia,Varanasi, Vol. II, PP. 569-571.

Sharma PV. (1996), Classical Uses of Medicinal Plants, Chowkhambha Bharati Academy, Varanasi.

Sharma PV. (1999), “Susruta Samhita”, Chaukhambha Vishvabharati, Varanasi, Vol.I.

Sharma PV. (2000), “Charaka Samhita”, Chaukhambha Oriantalia Varanasi, Vol. 2, Ci 2/1/28; 2/1/33; 2/2/4; 2/2/14; 2/3/15; 2/4/23; 2/4/28 .

Sharma PV. (2000), “Susruta Samhita”, Chaukhambha Vishvabharati, Varanasi, Vol. II.

Sharma PV. (2004), “Priyanighantuh”, Chokhambha Sanskrit Pratishthan, Varanasi, PP-66.

Shastri RD. (1985), “Bhaishajya Ratnavali”, Chowkhamba Saskrit Sansthan, Varanasi.

Shri Vaidya Shankarlalji Jain (1996), “Vanga Sen Samhita” : Vanga Sen Samhitan with hindi commentary of Shri Shaligramji Vaishya, edited by Khemraj Shri Krishnadas Prakashan, Bombay, PP.725-26, Karna Rogadhikar, Shloka no.5.

Shukla KK, Mahdi AA, Ahmad MK, Jaiswar SP, Shankwar SN, Tiwari SC. (2007), *Mucuna pruriens* reduces stress and improves the quality of semen in infertile men. *Evidence Based Complementernative and Alternative Medicine*, **7(1)**: 137-144.

Shukla KK, Mahdi AA, Ahmad MK, Shankhwar SN, Rajender S, Jaiswar SP. (2009), *Mucuna pruriens* improves male fertility by its action on the hypothalamus pituitary gonadal axis. *Fertil Steril*., **92(6)**: 1934-40.

Siddhuraju P, Becker K, Makkar HPS. (2000), Studies on the nutritional composition and antinutritional factors of three different seed material of an under-utilised tropical legume, *Mucuna pruriens var. utilis*. *J. Agric. Food Chem*. **48**: 6048-6060.

Siddhuraju P, Becker K. (2001), Rapid reversed-phase High Performance Liquid Chromatographic method for the quantification of L-Dopa (L-3, 4-dihydroxyphenylalanine), non-methylated and methylated tetrahydroisoquinoline compounds from *Mucuna* beans. *Food Chem*. **72**: 389–394.

Siddhuraju P, Vijayakumari K, Janardhanan K. (1996) Chemical composition and protein quality of the little-known legume, velvet bean (*Mucuna pruriens* (L.) DC.). *J. Agric. Food Chem*., **44**: 2636-2641.

Siddiqui AA, Ali M. (1997), Practical Pharmaceutical Chemistry. 1st edn; CBS Publishers and distributors, New Delhi, PP. 126-131.

Singh AP, Sarkar S, Tripathi M, Rajender S. (2013), *Mucuna pruriens* and its Major Constituent L-DOPA Recover Spermatogenic Loss by Combating ROS, Loss of Mitochondrial Membrane Potential and Apoptosis. *Advanced search*, **1**: 8.

Singh NP, Karthikeyan S (2000), *Flora of Maharashtra State Dicotyledon*. Botanical Survey of India, Calcutta.

Singh AK, Singh M, Singh R, Kumar S, Kalloo G. (2006), Genetic diversity within the genus *Solanum* (Solanaceae) as revealed by RAPD markers. *Current Science*, **90:** 711-716.

Singh N, Singh M, Kumar S, Kumar R, Singh V, Prasanna HC, Mathura R. (2007), RAPD markers for hybrid seed purity testing in tomato (*Solanum lycopersicum* L.). *Current Science*, **93**: 462-463.

Slinkard K, Singleton VL. (1977), Total phenol analyses; automation and comparison with manual methods. *Am. J. Enol. Vitic*., **28**, 49-55.

Sri Indradeva Tripathi (2011), edn Sri Gangasahaya Pandeya "Gadanigraha" of Sri Vaidya Sodhala, Part-2, Kayachikitsa Khanda, Chaukhambha Sanskrut Sasthan Varanasi.

Srikantha Murthy (1998), "Bhavaprakasa" of Bhavamisra, Krishnadas Academy, Varanasi, Vol. 1, Ni. Guduchydi varga-57, 6/4-129/131.

Srikantha Murthy (2000), Vagbhata's "Astanga Hrdayam", Krishnadas Academy, Varanasi, Vol. 3, U 40-17,24,31,34 .

Srikantha Murthy (2001), Vagbhata's "Astanga Sangraha", Chaukhambha Orientalia, Varanasi, Vol. 1, Su 7-31; 29-11,Ni 13-40; Chi 5-53,69; 8-19, 9-125, 23-72,86; Ka 5-23,34,49; U 1-18,9-30,32; 28-46,43.

Srinivasrao P. (2010), "Bhela Samhita", Chowkhambha Krishnadas Academy Varanasi.

Subramaniam P, Sunil Kumar (2001), Physicochemical profile of some colouring plants used in Homeopathy. *Ancient Science of life*, **21(2)**: 1-8.

Suresh S, Prithiviraj E, Prakash S. (2009), Dose and time dependent effects of ethanolic extract of *Mucuna pruriens* Linn. seed on sexual behaviour of normal male rats. *J Ethnopharmacol.*, **122(3)**: 497-501.

Tan NH, Fung SY, Sim SM, Marinello E, Guerranti R, Aguiyi JC. (2009), The protective effect of *Mucuna pruriens* seeds against snake venom poisoning. *J. Ethnopharmacol.*, **123(2)**: 356-358.

Thakur Balwant Singh, Chunekar KC. (1999), Glossary of Vegetable Drugs in Brhattrayi, Chaukhamba Amarbharati Prakashan, Varanasi, PP.72, 299, 350, 461.

Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. (2011), Phytochemical screening and extraction: A review. *International Pharmaceutical Science*, **1:** 98-106

Trease GE, Evans WC. (1989), Pharmacognosy. 11th edn; Brailliar Tiridel Can. Macmillian Publishers.

Tripathi J. (2012), “Chakradatta” of Sri Chakrapanidatta, Chowkhambha Sanskrit Series office, Varanasi.

Tripathi YB, Upadhyay AK. (2001), Antioxidant property of *Mucuna pruriens* Linn. *Current Science*, **80**: 1377-1378.

Udoh P, Ekpenyong J. (2001), Effect of *Mucuna urens* (horse eye bean) on the gonads of male guinea-pigs. *Phytotherapy Research*, **15(2)**: 99-102.

Ujowundu CO, Kalu FN, Emejulu AA, Okafor OE, Nkwonta CG, Nwosunjoku E. (2010), Evaluation of the chemical composition of *Mucuna utilis* leaves used in herbal medicine in Southeastern.Nigeria. *Afr. J. Pharm. Pharmacol*. **4**:811–816.

Ukachukwu SN, Obioha FC. (1997), Chemical evaluation of *Mucuna cochinchinensis* as alternatives protein feed stuff. *J Appl Chem Agric Res.*, **4**: 37-41.

Vadivel V, Janardhan K. (2000), Nutritional and antinutritional composition of velvet bean: an under-utilized food legume in South India. *International Journal of Food Science Nutrition*, **51**: 279-287.

Vaidya B (1998), “Nighantu Adarsha”, Chaukhambha Bharati Academy, Varanasi, (India), Palashadi Varga, Vol. 1, PP.453-456.

Vaidya B. (1982), Some controversial drugs in Indian medicine, Chaukhambha Orientalia, Varanasi (India). PP.247-248.

Vaidya BG. (1977), Glossary of Brihatrayi plants-4, Sachitra Ayurved, Vol-29(10), PP. 729-735.

Vaidya K, Kulkarni PH. (1991), A study of an Ayurvedic formula *viz*. Jivak. *Deerghayu International*, 7**(1):** 20.

Vaidya RA, Sheth AR, Aloorkar SD. (1978), The inhibitory effect of cowhage plant - *Mucuna pruriens* and L-DOPA on Chlorpromazine induced hyperprolactinemia in man. *Neurology* (India), **26:** 177-178.

Verma SC, Vashishth E, Singh R, Pant P, Padhi MM. (2014), A review on phytochemistry and pharmacological activity of parts of *Mucuna Pruriens* used as an Ayurvedic medicine. *World Journal of Pharmaceutical Research*, **3(5)**:138-158.

Vijayambika C, Jegadeesan M, Ganthi AS. (2011), Comparative anatomical studies on seeds of *Mucuna* Adans. and *Canavalia* DC. Species. *Indian Journal of Natural Products and Resources.* **2(1)**: 81-87.

Vishvanath Dwivedi Sastry (2007), "Bhavprakasa Nighantuh", Guduchyadi Varga, Chaukhambha Bharati Academy, Varanasi, PP. 197-198.

Wall ME, Krider MM, Krewson CF, Eddy CR, Willaman JJ, Correll DS, Gentry HS. (1954), Steroidal sapogenins – Survey of plants for steroidal sapogenins and other constituents VII. *Jour. Amer. Pharm. Ass., Sci Ed.*, **43**: 1-7.

Wichers HJ, Wijnsma R, Visser JF, Malingre TM, Huizing HJ. (1985), Production of L-DOPA by cell suspension cultures of *Mucuna pruriens.* (*II.* Effect of environmental parameters on the production of L-DOPA). *Plant Cell Tissue Organ Culture*, **4**: 75 - 82.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. (1990), DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* **18:** 6531-6535.

Wolfe AD, Liston A. (1998). Contributions of PCR-based methods to plant systematic and evolutionary biology. In plant molecular systematic. **2**: 55 - 63.

Yelne MB, Sharma PC. (1992), Pharmacognostic Study of Kapikacchu (*Mucuna pruriens* (L.) DC.) – Root and Seed. *Bulletin of Medico-Ethno-Botanical Research,* **XIII (1-2):** 52-64.

Yoganarasimhan SN. (1996), Medicinal Plants of India, Tamil Nadu. Interline Publishing Pvt. Ltd. Bangalore, India.

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Research Article

A COMPARATIVE ASSESSMENT OF PHARMACOLOGICALLY ACTIVE PRINCIPLES AND ANTIOXIDANT ACTIVITY OF COMMONLY OCCURRING *MUCUNA SPS.* IN INDIA

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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 (Leguminoceae) 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, anthelminitc, alexipharmic, hypotensive, spasmodic, hypocholestrolemic, 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, powered 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 stembark 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 [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50ºC 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 ($FeCl_3$) (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 *M 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.	*Mucuna cochinchinensis* black seed	190.7156 ± 4.2161
2.	*Mucuna cochinchinensis* white seed	96.2282 ± 3.9161
3.	*Mucuna pruriens*	77.6826 ± 3.1232
4.	*Mucuna bracteata*	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	*M. cochinchinensis* black seed extract	*M. cochinchinensis* white seed extract	*M. pruriens* extract	*M. bracteata* 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 spermatogensis 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 facililation 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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REFERENCES

1. Eilittä M, Bressani R, Carew LB, Carsky RJ, Flores M, Gilbert R, Huyck L, St-Laurent L, Szabo NJ. Mucuna as a food and feed crop: an overview. In: Mucuna as a Food and Feed: Current Uses and the

Way Forward. Ed. By Flores M, Eilittä M, Myhrman R, Carew L, Carsky R, Workshop held April 26-29, 2000 in Tegucigalpa, Honduras. CIDICCO Honduras. 2002; pp. 18-46.

2. Janardhanan K, Lakshmanan KK. Studies on the pulse, Mucuna utilis. Chemical composition and anti-nutritional factors. J. Food Sci. Tech. 1985; 22: 369-37.
3. Mohan VR, Janardhanan K. Studies on the Indian tribal pulses. In proceedings of the national seminar on biodiversity: strategies for conservation and future challenges. Department of Botany. Bharathiar University. Ed. by Udaiyan K, Janardhanan K, Manian S, Reddy VRKP 1993; pp. 116-125.
4. Manyam BV. An alternative medicine treatment for Parkinson's disease: results of a multicenter clinical trial – HP-200 in Parkinson's disease study group. J. Alternat. Comp. Med. 1995; 1, 249-255.
5. Standley PC, Steyermark JA. Flora of Guatemala. Fieldian: Botany, 1946; Vol 24 Part V.
6. Ding Y, Kinjo J, Yang C, Nohara T. Triterpenes from Mucuna birdwoddiana. Phytochemistry, 1991; 30(11), 3703-3707.
7. Sastry CST, Kavathekar YY. Plants for reclamation of wastelands. Publications and Information Directorate, New Delhi. 1990; pp 317-318.
8. Singh U, Wadhwani AM, Johri BM. Dictionary of economic plants in India. Indian Council of Agricultural Research, New Delhi. 1996; pp. 45-46.
9. Anonymous. The Wealth of India Raw Materials, NISCAIR Publishing New Delhi, L-M; VI, 2005; 439-444.
10. Desai SM., Sharon M, Sharan M. Effect of Culture Conditions on L-Dopa Accumulation in Callus Culture of Mucuna pruriens. J. Chem. Pharm. Res, 2010; 2(4), 134-146.
11. Lowry OH, Rosebrough NR, Lewis Farr A, Randall RJ. Protein measurement with the Folin Phenol Reagent, J. Biol. Chem, 1951; 193, 265-275.
12. Khatiwora E, Adsul VB, Kulkarni MK, Deshpande NR, Kashalkar RV. Spectroscopic determination of total phenol and flavonoid contents of Ipomoea carnea. International Journal of ChemTech Research, 2010; 2 (3), 1698-1701.
13. Slinkard K, Singleton V.L. Total phenol analyses; automation and comparison with manual methods. Am. J. Enol. Vitic. 1977; 28, 49-55.
14. Blois, M.S. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181, 1199-1200.
15. Oyaizu, M. Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition. 1986; 44, 307-315.
16. Daxenbichler ME, Van Etten CH, Hallman EA, Earle FR, Barclay AS. Seeds as sources of L-DOPA. J. Med. Chem. 1971; 14, 463.
17. British Pharmacopoeia. (1973), London.
18. Longhi JG, Perez E, Jose de Lima J, Candido LMB. In vitro evaluation of Mucuna pruriens (L.) DC. antioxidant activity. Brazilian Journal of Pharmaceutical Sciences, 2011; 47(3), 535-544.
19. Singh AP, Sarkar S, Tripathi M, Singh R. Mucuna pruriens and Its Major Constituent L-DOPA Recover Spermatogenic Loss by Combating ROS, Loss of Mitochondrial Membrane Potential and Apoptosis. PLoS One. 2013; 8(1), e54655.
20. Malmnas CO. The significance of dopamine, versus other catecholamines, for L-dopa induced facilitation of sexual behavior in the castrated male rat. Pharmacol. Biochem. Behav., 1976; 4(5), 521-526.
21. Gulcin I. (). Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa. Amino Acids, 2007; 32, 431–438.
22. Gow-Chin Y, Chiu-Luan H. Antioxidant effects of dopamine and related compounds. Biosci Biotech Biochem, 1997; 61 (10), 1646–1649.
23. Kazuki K, Hiroyuki S. High content of dopamine, a strong antioxidant, in Cavendish banana. J Agric Food Chem, 2000; 48, 844–848.

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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.

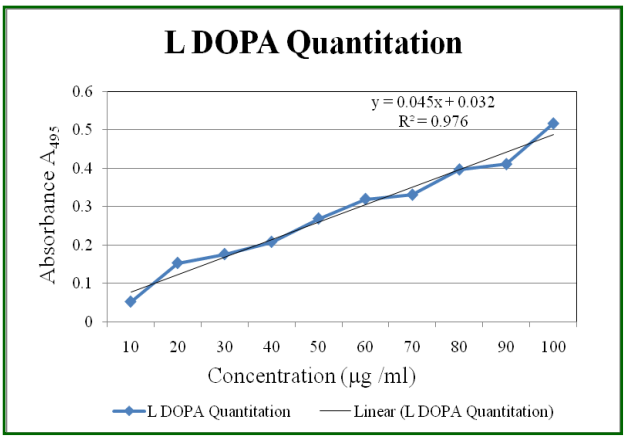


Figure a: The standard plot of L-dopamine

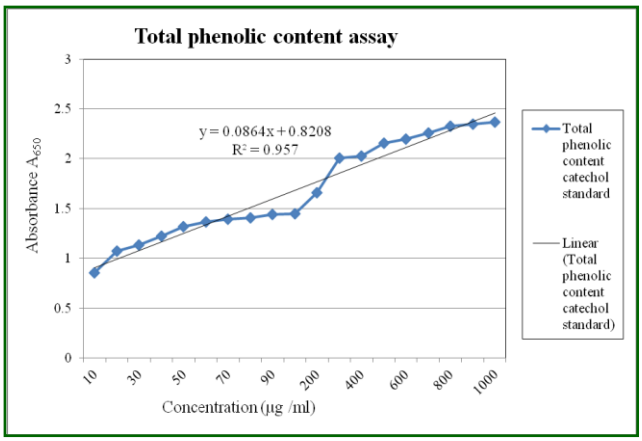


Figure b: The standard plot of total phenolic content assay

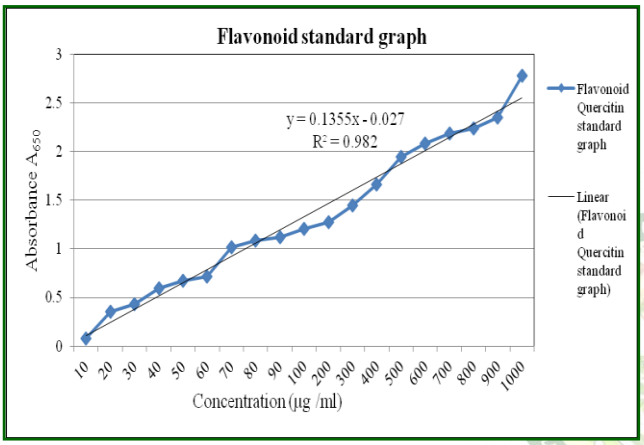


Figure c: The standard plot of flavonoid assay

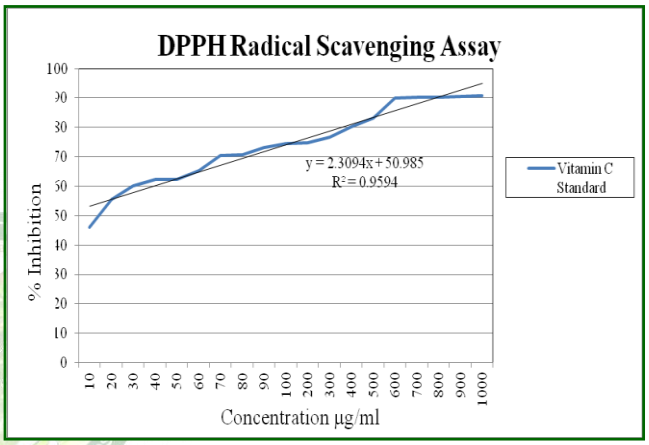


Figure d: The standard plot of DPPH radical scavenging assay

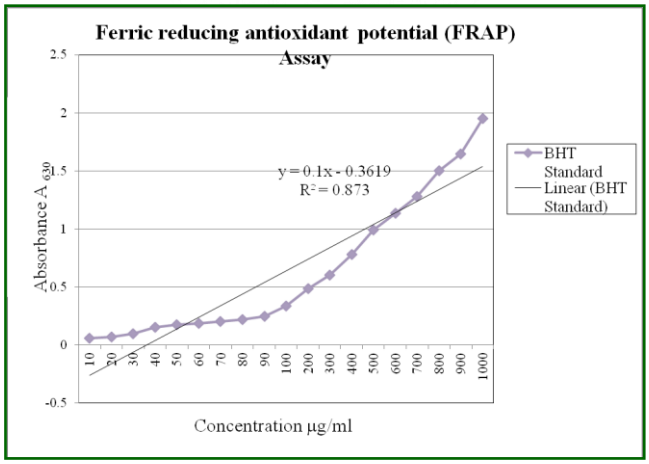


Figure e: The standard plot of FRAP assay

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Molecular Analysis of *Mucuna pruriens* (L.) DC. and *Mucuna cochinchinensis* (Lour.) A. Chev. Using RAPD Markers Helps to Understand Genetic Variations

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Abstract: *Fifteen species of Mucuna species are reported so far from India which are being used in the name of Kapikacchu by the ayurvedic practitioners since ancient times. Kapikacchu is an ingredient of several commercial preparations that are being used as an aphrodisiac in male sexual disorders. The samples of Mucuna pruriens (L.) DC. and Mucuna cochinchinensis (Lour.) A. Chev. were authenticated, analyzed and compared to illustrate the phylogenetic relationships between them. In this study, a set of 25 plants RAPD universal primers (RPI 1 – RPI 25) were used for genomic DNA amplification. The amplicons were compared and the phylogenetic tree was drawn with the help of bioinformatic analysis tool UPGMA (Unweighted Pair Group Method with Arithmetic Mean) by applying Jaccard's distance method. The Jaccard's similarity coefficient values suggested good variability in these two Mucuna sps. 107 RAPD fragments were obtained using twenty five primers. The RAPD PCR (Random Amplification of Polymorphic DNA- Polymerase chain reaction) amplification resulted in different successive polymorphic banding patterns with primers in both the plants that indicate significant genetic variations between the species. The creation of some baseline data is an important approach to study the genetic markers of Mucuna species as the information on the genetic diversity of Mucuna species is inadequate.*

Keywords*: Mucuna pruriens; Velvet bean; Mucuna cochinchinensis; Lyon bean; RAPD profiling; Genetic divergence; Molecular polymorphism*

I. Introduction

The genus *Mucuna* belongs to the family Fabaceae (Leguminoceae) and includes about 150 species of annual and perennial legumes of tropical distribution. India is one of the natural centers of origin of the *Mucuna* in the world (Eilittä *et al*., 2002). To date fifteen species of *Mucuna* species are reported from India. In addition to their traditional medicinal use many species of the genus offer an excellent source as cover crop and green manure (Janardhanan and Lakshmanan, 1985; Mohan and Janardhanan, 1993). 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 (Manyam, 1995). In addition, *Mucuna* is also traditionally used in various other applications like, dye (Standley and Steyermark, 1946), treatment of pain and numbness of joints, and irregular menstruation (Ding *et al*., 1991).

Mucuna pruriens Bak. 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 (Sastry and Kavathekar 1990; Singh *et al*., 1996). It is reported to be native of China and Eastern India (Wilmot Dear, 1987). M. pruriens is a wild plant and it's every part is full of medicinal value. It's most important parts are seeds and roots which are good source of giving vital energies. Seeds are excellent source of L-DOPA (lavodopa 3,4- dihydroxyphenyl alanine) which is precursor of dopamine a neurotransmitter (Daxenbichler *et al*.,1971) used in the treatment of Parkinson's disease (British pharmacopoeia, 1973; Longhi *et al*., 2011). The seed of *M. cochienchinensis* contains carbohydrate 55.8%, protein 27.5% and fat 3.6%. The fruits yield 0.96% L-dopa (Anonymous, 2006)**.**

Mucuna cochinchinensis (Lour.) A. Chev., 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 (Anonymous, 2005).

Randomly Amplified Polymorphic DNA (RAPD) is convenient technique to analyse the genetic variation (Bhat *et al*., 1999). It has been extensively used for molecular fingerprinting and population diversity analysis (Albert *et al*., 1997, Saini *et al*., 2010). Availability of reliable polymorphic markers, often limits the accurate estimation of genetic variation among individuals or different populations.

In the present study, we have applied a DNA fingerprinting technique, random amplified polymorphic DNA (RAPD), to differentiate the three accessions of *Mucuna* as well as for genetic characterization of the selected accession.

II. Material And Methods

Collection of Plant Materials:

The leaves of *Mucuna pruriens* (L.) DC. and *Mucuna cochinchinensis* (Lour.) A. Chev. were collected from the Medicinal plants garden of National Research Ayurvedic Institute of Basic Ayurvedic Sciences, Pune. The plant materials were authenticated by Mrs. A. G. Mhase, Herbarium Assistant, and the specimens were preserved in the herbarium for reference.

DNA Extraction:

For RAPD analysis, healthy and non-infected leaves were collected from mother plant. The leaves samples were grounded in liquid nitrogen to a fine powder and were kept at -20°C until use**.** The total genomic DNA extraction from the leaves of both the plants was done by using 3B Black Biotech Biotools kit with approximately 100-120 mg of powdered sample of each. The DNA of both plants was then quantified using biophotometer (eppendorf) as well as standardized against known concentrations of DNA marker ladder (Promega, USA) on 1% agarose gels. DNA was then kept at 4°C until further use.

RAPD-PCR:

The protocol for polymerase chain reaction (PCR) was optimized by varying the concentration of template, *Taq* DNA polymerase and $MgCl_2$. Polymerase chain reaction (PCR) amplifications were performed in reaction mixtures containing 10 ng template DNA, 0.2 µM primer, 100 µM dNTP´s, 10 mM PCR buffer, 1.5 mM $MgCl_2$, and 1 unit *Taq*-polymerase in a total volume of 20 µl.

Amplifications were carried out in a thermo-cycler (Peltier P25+, Cyber lab) with the following program: initial denaturation step of 3 min at 94°C followed by 35 cycles of 45 sec at 94°C, 30 sec at 36°C, 1 min at 72°C, and a final extension step of 5 min at 72°C and 4ºC for the end hold. PCR products were resolved by electrophoresis in 1.5% agarose gels along with DNA Marker ladder (100-1000bp), the run was carried out at 75 Volts for approximately 60 minutes. Gels were stained with ethidium bromide, visualized under UV-light, and photographed GelDoc system (BioRad, USA). To ensure reproducibility of banding patterns, each amplification reaction was repeated twice. All the PCR reaction components and the Primers set were purchased from 3B Blackbio Biotech Biotools. The primers used for amplification of the genomic DNA are detailed in Table 1. Along with the 25 different reaction mixtures for 25 different primers, a negative control (NC - No DNA template) and a positive control (PC - No primer) were placed in thermal cycler to rule out the possibility of contamination. Reaction mixture wherein template DNA replaced by distilled water was used as negative control.

Table 1: Plant RAPD primers used for the study

No.	Primer code	Accession Numbers
1	RPl 1	AM765819
2	RPl 2	AM750044
3	RPl 3	AM773310
4	RPl 4	AM773769
5	RPl 5	AM773770
6	RPl 6	AM773771
7	RPl 7	AM773312
8	RPl 8	AM773773
9	RPl 9	AM773315
10	RPl 10	AM750045
11	RPl 11	AM911709
12	RPl 12	AM773316
13	RPl 13	AM750046
14	RPl 14	AM773774
15	RPl 15	AM773775
16	RPl 16	AM773776
17	RPl 17	AM911710
18	RPl 18	AM765830
19	RPl 19	AM773777
20	RPl 20	AM773317
21	RPl 21	AM765820
22	RPl 22	AM911711
23	RPl 23	AM911712
24	RPl 24	AM765821
25	RPl 25	AM750054

Statistical analysis:

Gelquest® and Clustervis® software were used to construct dendro-grams using the Unweighted Pair Group Method with Arithmetical Averages (UPGMA) by comparing the bands that were observed common in both plants to specific primers.

III. Results

The DNA obtained was 21.32 ng/µl (*M. pruriens*) and 20.5ng/µl (*M. cochinchinensis*).

As evident from RAPD profiles, the two accessions selected of *Mucuna* could be distinguished at the molecular level. The comparative analysis of all accessions was established on the basis of RAPD profiles generated by using decanucleotide primers. Figure 1 shows the characteristic RAPD profile of *M. pruriens*, whereas Figure 2 clearly depicts the genetic differentiation of *M. cochinchinensis*. Only the primers which displayed prominent, reproducible and distinguishable bands were considered for analysis. The 14 tested primers yielded totally 107 scorable bands with an average of three bands per primer which showed polymorphism. 60% polymorphism was observed in *M. cochinchinensis* and 92% in M. pruriens. All primers showed amplification for *M. pruriens* whereas primer number 4, 18 and 24 did not show any bands in the RAPD profile of *M. cochinchinensis.*

Figure 1: RAPD ***Mucuna* pruriens (L.) DC.**

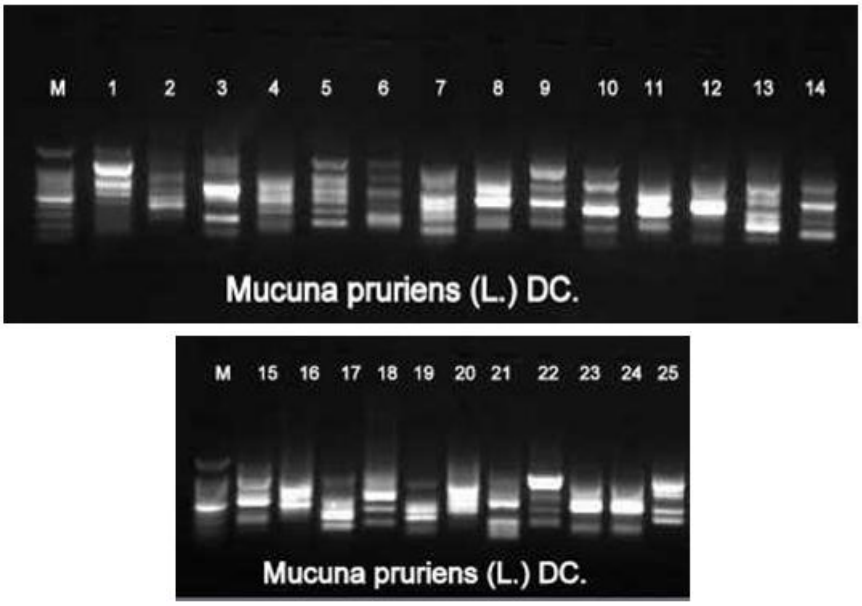
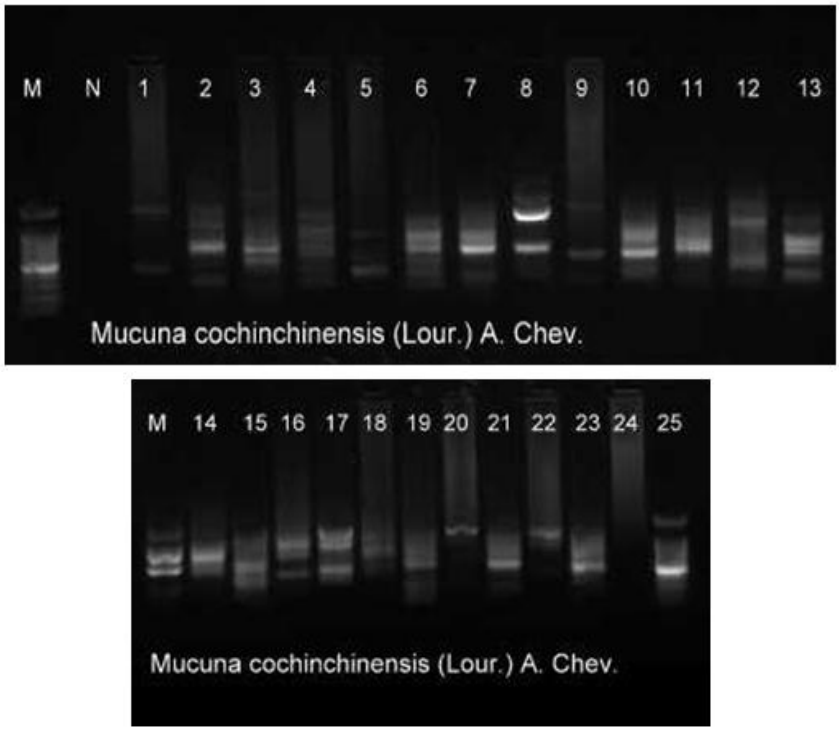
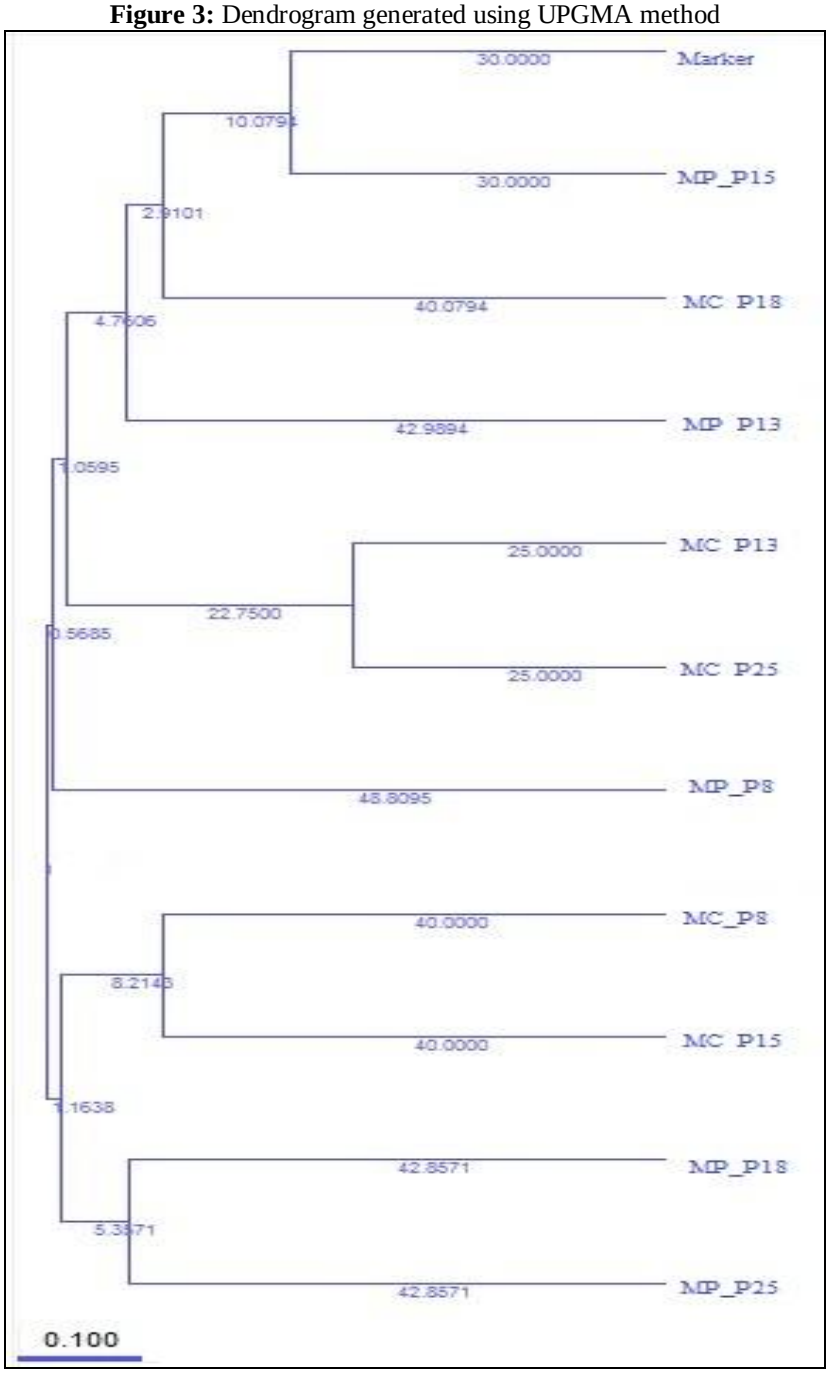


Figure 2: *Mucuna* cochinchinensis (Lour.) A. Chev.



The data scored on RAPD polymorphism and band sharing was aimed at analyzing the genetic similarity matrix obtained through multivariate analysis using Nei and Li's coefficient. The similarity coefficients were used to generate a tree for cluster analysis using the UPGMA method as evident from the phenogram (Figure 3).

Figure 3: Dendrogram generated using UPGMA method



IV. Discussion

RAPD analysis in the present study reveled very high level of Polymorphism (92%) for different *Mucuna* species. Padmesh *et al*., (2006) observed that 11 *Mucuna* accessions produced 90.1% polymorphism. Leelambika *et al*., (2010), observed 98% polymorphism in 18 accessions of *Mucuna*. Generated data showed RAPD markers are suitable tools to scan anonymous DNA of species with high secondary metabolite content such as *Mucuna*. The limited reliability of RAPD molecular markers was compensated by increasing the number of RAPD fragments. In this study, the large similarity values revealed by RAPD markers while making the phylogenetic tree provide greater confidence for assessment of genetic relationship among the species. RAPD profiling of these two plants have not yet reported.

Hadrys *et al*., (1992) used RAPD-PCR technique for studying genotype fusion at various levels, including those of individuals (parentage tests) and populations (identification of hybrid populations, species and subspecies). This profiling method was used for isolating soma-clone of altered genotypes in *Mentha arvensis* by Khanuja *et al*., 1998. The UPGMA tree showed that the two plants were related and have the common ancestral background. Though these two have different uses in Ayurvedic sciences they still can be tracked back. Based on the study the large range of similarity and dissimilarity values for the plants provides the greater confidence for assessment of genetic diversity and relationships.

V. Conclusion

The information on genetic diversity of *Mucuna* species evaluated using RAPD technique and the results provided a short and snappy view on the total genetic variability for *Mucuna* species. RAPD fingerprinting analysis is widely used in analyzing population and genetic relationships between and among species, establishing taxonomic status and evolutionary studies. Thus, this approach will be helpful in ranking the species according to their genetic inter-relatedness. This can provide a better platform for identification and authentication of *Mucuna* species. Therefore this study may contribute to provide germplasm conservationists with data in order to facilitate conservation efforts. In summary, the analysis of amplification spectra of 107 bands obtained by 25 primers clearly demonstrated the differentiation of selected accessions and established the effectiveness of this molecular tool for establishing the distinct identity of selected accessions of *Mucuna*.

Acknowledgments

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References

[1]. Albert, M.J., Bhuiyan, N.A., Talukder, K.A., Faruque, A.S.G., Nahar, S., Faruque, S.M., Ansaruzzaman, M. and Rahman, M. (1997). Phenotypic and genotypic changes in Vibrio cholerae 0139 Bengal. J. Clini. Microbio, **35**: 2588–2592.
[2]. Anonymous. (2005). The Wealth of India Raw Materials, NISCAIR Publishing New Delhi, L-M; **VI**: 439-444.
[3]. Anonymous. (2006). The Wealth of India- Raw Materials. First Suppl Ser. NISCAIR; 166-167.
[4]. Bhat, K.V., Babrekar, P.P. and Lakhanpaul, S. (1999). Study of genetic diversity in Indian and exotic sesame (*Sesamum indicum* L.) germplasm using random amplified polymorphic DNA (RAPD) markers. Euphytica, **110**: 21-34.
[5]. British Pharmacopoeia. (1973), London.
[6]. Daxenbichler, M.E., Van Etten, C.H., Hallman, E.A., Earle, F.R. and Barclay, A.S. (1971). Seeds as sources of L-DOPA. J. Med. Chem. **14**: 463.
[7]. Ding, Y., Kinjo, J., Yang, C. and Nohara, T. (1991). Triterpenes from *Mucuna* birdwoddiana. Phytochemistry **30(11)**: 3703-3707.
[8]. Eilittä, M., Bressani, R., Carew, L.B., Carsky, R.J., Flores, M., Gilbert, R., Huyck, L., St-Laurent, L. and Szabo, N.J. (2002). *Mucuna* as a food and feed crop: an overview. In: *Mucuna* as a Food and Feed: Current Uses and the Way Forward. Ed. By Flores M, Eilittä M, Myhrman R, Carew L, Carsky R, Workshop held April 26-29, 2000 in Tegucigalpa, Honduras. CIDICCO Honduras. pp. 18-46.
[9]. Hadrys, H., Balick, M. and Schierwater, B. (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol. Ecol. **1**: 55-63.
[10]. Janardhanan K. and Lakshmanan K.K. (1985). Studies on the pulse, *Mucuna* utilis. Chemical composition and anti-nutritional factors. J. Food Sci. Tech. 22: 369-37.
[11]. Khanuja, S.P.S., Shasany, A.K., Dhawan, S. and Kumar, S. (1998). Rapid procedure for isolating somaclones of altered genotypes in *Mentha arvensis*. J. Med. Arom. Plant Sci. **20**: 359-361.
[12]. Leelambika, M., Mahesh, S., Jaheer, M. and Sathyanarayana, N. (2010). Comparative evaluation of genetic diversity among Indian *Mucuna* species using morphometric, biochemical and molecular approaches. World Journal of Agricultural Sciences, **6(5):** 568-578.
[13]. Longhi, J.G., Perez, E., Jose de Lima, J. and Candido L.M.B. (2011). In vitro evaluation of *Mucuna pruriens* (L.) DC. antioxidant activity. Brazilian Journal of Pharmaceutical Sciences, **47(3)**: 535-544.
[14]. Manyam, B.V. (1995). An alternative medicine treatment for Parkinson's disease: results of a multicenter clinical trial – HP-200 in Parkinson's disease study group. J. Alternat. Comp. Med. **1**: 249-255.
[15]. Mohan, V.R. and Janardhanan, K. (1993). Studies on the Indian tribal pulses. In proceedings of the national seminar on biodiversity: strategies for conservation and future challenges. Department of Botany. Bharathiar University. Ed. by Udaiyan K, Janardhanan K, Manian S, Reddy VRKP. pp. 116-125.
[16]. Padmesh, P., Reji, J.V., Dhar, M. and Seeni, S. (2006). Estimation of genetic diversity in varieties of *Mucuna pruriens* using RAPD. Biologia plantarum, **50:**367-372.
[17]. Saini, M., Singh, S., Hussain, Z. and Yadav, A. (2010). RAPD analysis in mungbean [*Vigna radiata* (L.) Wilczek]: I. Assessment of genetic diversity. Indian J. Biotech, **9**: 137 –146.
[18]. Sastry, C.S.T. and Kavathekar, Y.Y. (1990). Plants for reclamation of wastelands. Publications and Information Directorate, New Delhi. p.317-318.
[19]. Singh, U., Wadhwani, A.M. and Johri, B.M. (1996). Dictionary of economic plants in India. Indian Council of Agricultural Research, New Delhi. p. 45-46.
[20]. Standley, P.C. and Steyermark, J.A. (1946). Flora of Guatemala. Fieldian: Botany Vol **24** Part **V.**
[21]. Wilmot Dear C.M. (1987). A revision of *Mucuna* (Leguminosae - Phaseolae) in the Indian Subcontinent and Burma. Kew Bull., **42(1)**: 23–46.

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In vitro Physico-chemical, Phytochemical and Fluroscence Assessment of Mucuna sps.

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Abstract: *Mucuna is commonly called as "Kapikacchu', which is known to have various medicinal properties and being used in Ayurveda since ages. Medicinal plants have bioactive compounds which are used for curing various diseases. The present investigation is aimed to screen the various bioactive compounds present in seeds of Mucuna pruriens (L.) DC., Mucuna cochinchinensis (Lour.)A. Chev. black seeds variety and Mucuna cochinchinensis (Lour.) A. Chev. white seeds variety. In the present study, an attempt was made to analyse and evaluate the presence of various phytoconstituents as well as fluorescent characteristics of seeds of Mucuna sps. Along with this, during the research work, physicochemical parameters were also determined. Qualitative phytochemical analysis of the aqueous and methanolic extracts of seeds showed the presence of various phytoconstituents i.e. carbohydrates, reducing sugar, hexose sugar, proteins, amino acids, steroids, saponins, flavonoids, alkaloids, tannins and phenols. Qualitative analysis of the aqueous extract was carried out. It was observed that all the extracts show more important chemical constituents for various pharmacological activities. In fluorescent analysis characteristic colours were observed with different chemical reagents in seed powder extracts under visible and UV light (short and long wavelength). The determination of these characters will aid future investigators in their Pharmacological analysis of this species. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases.*

Keywords: *Kapikacchu, Fluorescence, Physicochemical analysis, Mucuna pruriens (L.) DC., Mucuna cochinchinensis (Lour.)A. Chev.*

I. Introduction

Kapikacchu (Mucuna belongs to the family Fabaceae) is an ingredient of several commercial preparations that are being used as an aphrodisiac, in male sexual disorders. Seeds are astringent, laxative, anthelminitc, alexipharmic, hypotensive, spasmodic, hypocholestrolemic, 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 [1], treatment of pain and numbness of joints, and irregular menstruation [2].

The genus Mucuna belongs to the family Fabaceae (Leguminoceae) 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 [3]. 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 [4, 5]. Almost all the species are reported to be used in the treatment of Parkinson's disease [6].

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 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 commonly occurring Mucuna sps in India.

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]. It is reported to be native of China and Eastern India [9]. M. pruriens is a wild plant and it's every part is full of medicinal value. It's most important parts are seeds and roots which are good source of giving vital energies. Seeds are excellent source of L-DOPA (lavodopa 3,4- dihydroxyphenyl alanine) which is precursor of dopamine a neurotransmitter [10] used in the treatment of Parkinson's disease [11,12].

Mucuna cochinchinensis (Lour.)A. Chev. 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 [13]. The seeds of M. cochienchinensis contains carbohydrate 55.8%, protein 27.5% and fat 3.6%. The fruits yield 0.96% L-dopa [14].

The present study was aimed to assess and compare the physicochemical and phytochemical (qualitative as well as quantitative) parameters of Mucuna pruriens (L.) DC., Mucuna cochinchinensis (Lour.)A. Chev. black seeds variety and Mucuna cochinchinensis (Lour.) A. Chev. white seeds variety.

II. Materials and Methods

1. Collection and Processing

Seeds of Mucuna pruriens (L.) DC., Mucuna cochinchinensis (Lour.)A. Chev. black seeds variety and Mucuna cochinchinensis (Lour.) A. Chev. white seeds variety were collected from Medicinal Plant Garden of National Research Institute of Basic Ayurvedic Sciences, Kothrud, Pune, India during the month of January - February, 2013. Samples were taxonomically identified in the institute and were then deposited in the herbarium section for future reference. Samples were taxonomically authenticated by Plant Sciences Division of Agharkar Research Institute, Pune with voucher numbers S-159 (Mucuna cochinchinensis (Lour.)A. Chev. black seeds variety), S-161 (Mucuna cochinchinensis (Lour.)A. Chev. white seeds variety), and S-162 (Mucuna pruriens (L.) DC.).

2. Physicochemical Parameters

Physicochemical parameters like Soluble extractive values of dried seeds powder in Absolute Alcohol, Water, Methanol, Acetone, Ethyl acetate, Petroleum ether, Benzene, Chloroform, Carbon tetrachloride, Hydro-alcoholic; Total ash; Acid insoluble ash; Water soluble ash, Loss on drying (LOD); Foreign matter; Swelling factor, Specific gravity and pH value were determined using the standard protocols and guidelines.[15-17]

3. Extracts Preparation for Phytochemical analysis

Extraction of dried plant material was carried out using a method described by Harborne (1973) with minor modifications. The leaves were thoroughly washed under tap water followed by washing with distilled water.[18] Collected seeds as well as washed leaves were shade dried and powdered. The powdered leaves and seeds were kept overnight in different solvents systems like water (aqueous), methanol and hydro-alcohol. Extracts were filtered using Whatmann no. 42 (125mm) filter paper. The extracts were evaporated to reduce the volume to half (approximately) on a water-bath for 1-2 hrs. The plant extracts were cooled and then a part of the extract was used directly for qualitative determination of phytochemical parameters and remaining extract was subjected to lyophilization which was further taken to evaluate the quantitative parameters.

4. Qualitative Phytochemical Analysis

The following tests were carried out to determine the presence of various active phyto-constituents.

Carbohydrates: To 2-3 ml extract, few drops of Molisch reagent was added, mixed well and conc. H_2SO_4 was added from the sides of the test tube, violet ring formation at the junction of two liquids indicated the presence of carbohydrates.[19]

Sugars: Presence of Reducing sugar, Pentose sugar and Hexose sugar were determined using the Fehling's, Aniline acetate and Cobalt Chloride test respectively as described by Sadasivam and Manickam (1996) and Kokate (1996) [20,21].

Amino acids: Ninhydrin test was conducted for amino acids in general and presence of cysteine was checked by adding 40% NaOH and 10% lead acetate solution to extract. Appearance of black lead sulphate precipitate after boiling confirmed the test [20].

Proteins: Presence of proteins was determined by Biuret test, Million's test, Xanthoprotein test, heat test, Lead acetate test and Ammonium sulphate test [20].

Steroids: Salkowski test and Libermann-Buchard test were conducted for assessing the presence of steroids. While performing Libermann-Buchard test to 2 ml extract with chloroform.1-2 ml aceteic unhydride few drops cons. H_2SO_4 was added from the side of test tube. Steroids were indicated by reddish brown coloured ring at the junction of two layers [22].

Sakowski test: To 2 ml of extract 2 ml chloroform and 2 ml of conc. H_2SO_4 was added and mixed well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence [21].

Glycosides: To 2 ml extract glacial acetic acid, few drops of 5% $FeCl_3$ and conc. H_2SO_4 were added. Reddish brown colour at the junction of two liquid layers and upper layer appears bluish green indicating the presence of glycosides.[23,24] Presence of Cardiac Glycosides, Anthroquinone glycosides [25,26] and Coumarin glycosides[27] were determined using the protocols described by Sadasivam and Manickam (1996) and Kokate (1996) [20,21].

Saponins: Foam and lead acetate tests were carried out for indicating the presence of saponins. The layer of foam formation on vigorous shaking of extract and distilled water confirmed the presence of saponins in foam test and formation of white precipitate on the addition of lead acetate indicated the positive saponin test [28, 29].

Flavonoids: Shinoda test, Lead acetate test and alkaline reagent tests were conducted for the flavonoid determination [21,28-30].
Shinoda Test: An alcoholic extract of plant material was treated with magnesium (dust) and conc. HCl. The appearance of Crimson red colour indicated the presence of flavonoids.
Lead Acetate test: To the small quantity of extract, lead acetate solution was added then yellow or reddish brown coloured precipitate determined the presence of flavonoids.
Alkaline Reagent test: The test solution was treated with sodium hydroxide which showed yellow coloration, indicating the presence of flavonoids.
Alkaloids: For the confirmation of alkaloids three tests were conducted [20, 21, 28].
Dragendroff's test confirmed the presence with formation of orange brown precipitate on addition of few drops of dragendroff's reagent onto the extract.
Mayer's test: Few drops of the Mayer's reagent was when added onto the extract, white - pale yellow precipitate was seen which indicated the presence of alkaloids.
Wagner's test**:** Extract was acidified with 1.5 % v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicated the presence of alkaloids.
Tannins: Lead acetate test was performed for confirming the presence of tannins in the extract. To an aqueous solution of dried plant material, 5% lead acetate solution was added; appearance of white precipitate indicated the presence of tannins [20, 28, 31].
Mucilage: Mucilage test was conducted as per the procols described in Kokate (1996). Powered drug material showed red color with ruthenium red [21].
Phenol: For the purpose of analysing the presence of phenol $FeCl_3$ test and Folin-ciocaltaue test was carried out [21, 32].
$FeCl_3$ Test: Small amount of extract of plant material was treated with neutral $FeCl_3$. Formation of blue or green colour indicated the presence of phenol [31].
Folin-ciocaltaue reagent test: When the extract was treated with a little residue of ammonia solution, a blue or blue to gray colour is formed indicating the phenols are present.
Starch: Iodine test was conducted for confirming the presence of starch in the extract. To 3 ml of extract few drops of dilute iodine solution was added and mixed. Appearance of blue colour which disappears on boiling and reappears on cooling indicated the presence of starch [20, 21].

5. Quantitative Phytochemical Analysis

For Quantification of various phytochemical constituents' standard procedures for estimation were performed. The parameters like Carbohydrates [16]; Flavanoids [33-35]; Tannins [35]; Proteins [36]; Glycosides [23,36]; Lipids [37] and Total Phenol Content[32,38] were quantified for all the Mucuna sps under study.

6. Fluorescence analysis of Powder

Fluorescence analysis of the powder was carried out as described by Nanna et al., 2013 and Birajdar et al., 2014 [39,40]. Seed and leaf powder were taken in different tubes. To each of these a fixed volume of different organic solvents like distilled water, ethanol, benzene, chloroform, methanol, glacial acetic acid, sulphuric acid, nitric acid, hydrochloric acid and ammonia were added. Then all the tubes were observed under the visible and UV light after shaking for the visualization of characteristic colour reaction were compared with a standard colour chart and colours were noted [41].

III. Results

This study revealed the presence of physicochemicals and phytochemicals considered as active medicinal chemical constituents. The physicochemical constituents of Mucuna sps are presented in Table 1. Total ash was highest in M. pruriens (3.88%) Physicochemical parameter analysis is studied for understanding the nutritional value, quality and microbiological stability of the drug. Swelling factor was not found to be present in M. pruriens. Table 2, represents the specific gravity of Mucuna sps extract with different solvents. These results show that all the species almost same specific gravity in each solvent.

Extractive values for Mucuna sps in different solvents were determined and depicted in Table 3. The comparison between different solvents and Mucuna sps showed that seed powder has significant extractive values in water, methanol and hydro-alcohol extracts.

In qualitative analysis of Mucuna sps it was found that, methanolic and water extracts showed the presence of alkaloids (Wagner's test) and phenols. When protein presence was checked using lead acetate test only methanolic extract gave positive detection. Hexose sugar was found only in methanolic extract of M. pruriens. Carbohydrates, reducing sugar, saponins, flavonoids (lead acetate test), alkaloids (Mayer's test) and starch was detected when water extract of the samples were analyzed. Volatile oil, organic acids, mucilage glycosides, pentose sugar and Cysteine amino acid was not detected in any of the sample and extract (Table 4).

The screening and quantitative analysis using water extract of Mucuna sps for the carbohydrates, flavonoids, tannins, lipids, proteins, total phenolic content and glycosides is presented and expressed in Table 5 followed by respective graphs (Fig. 1-7).

The results of the quantitative phytochemical analysis showed that the seeds M. cochienchinensis white seed variety are rich in carbohydrates, flavonoids, and tannins whereas M. cochienchinensis black seed variety showed significant quantity of proteins and total phenolic contents. In the quantification of lipid content and glycosides (though the amount was significantly low in all the sps under study) were present in maximum amount in M. pruriens.

The results of fluorescence analysis of Mucuna seeds with different solvents are expressed in Table 6. Fluorescence study is an essential and required parameter for first line standardization of crude drug. In this analysis most of the reactions resulted in colourless reaction mixture.

IV. Tables

Table 1: Physicochemical constituents of Mucuna sps

Sr. No.	Parameters	Mucuna pruriens (L.) DC.	Mucuna cochinchinensis (Lour.) A. Chev. (White Seed)	Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed)
1	pH	7.40	7.65	7.43
2	Loss on Drying (LOD)	5.56 %	10.15 %	9.29 %
3	Total Ash	3.88 %	3.42 %	3.53 %
4	Acid Insoluble Ash	0.09 %	0.04 %	0.10 %
5	Water soluble Ash	0.14 %	0.05 %	0.07 %
6	Swelling factor	0.0 ml	1.0 ml	1.0 ml

Table 2: Specific gravity of Mucuna species extract with different solvents

Sr. No.	Solvents	Mucuna pruriens (L.) DC.	Mucuna cochinchinensis (Lour.) A. Chev. (White Seed)	Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed)
1	Water	0.9831	0.9698	0.9739
2	Methanol	0.7801	0.5151	0.7669
3	Ethanol	0.7773	0.8160	0..7655
4	Acetone	0.7719	0.7935	0.7586
5	Ethyl acetate	0.8851	0.9001	0.8700
6	Petroleum ether	0.6518	0.6607	0.6452
7	Benzene	0.8570	0.8738	0.8574
8	Chloroform	1.4539	1.4454	1.4266
9	Carbon tetra chloride	1.5526	1.5521	1.5176

Table 3: Determination of Extractive values

Sr. No.	Extract	Mucuna pruriens (L.) DC. (in %)	Mucuna cochinchinensis (Lour.) A. Chev. (White Seed) (in %)	Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed) (in %)
1	Absolute alcohol	9.61	13.46	14.08
2	Water	29.00	21.89	24.82
3	Methanol	19.05	12.32	15.83
4	Acetone	7.41	6.07	10.74
5	Ethyl acetate	5.02	5.17	9.60
6	Petroleum ether	5.34	5.17	11.41
7	Benzene	5.84	5.63	11.05
8	Chloroform	8.20	8.22	15.05
9	Carbon tetra chloride	8.20	7.61	11.12
10	Hydroalcoholic (1:1)	38.64	38.13	38.05

Table 4: Qualitative analysis for Phytochemicals

Sr. No.	Test	Test Method	Mucuna pruriens (L.) DC.		Mucuna cochinchinensis (Lour.) A. Chev. (White Seed)		Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed)	
			ME	WE	ME	WE	ME	WE
1	Carbohydrates	Molish's test	-	+	-	+	-	+
2	Reducing sugar	Fehling's test	-	+	-	+	-	+
3	Pentose sugar	Phloroglucinol reag. Test	-	-	-	-	-	-
4	Hexose sugar	Tollen's Phloroglucinol test	+	-	-	-	-	-
5	Proteins	Biuret test	-	-	-	-	-	-
		Millon's test	-	-	-	-	-	-
		Xanthoprotein test	-	-	-	-	-	-
		Heat test	-	-	-	-	-	-
		5% Lead acetate	+	-	+	-	+	-
		5% Ammonium sulphate	-	+	-	-	-	-
6	Amino acid	a)Ninhydrin test	+	+	+	+	+	+
		b)Test for cysteine	-	-	-	-	-	-
7	Steroids	a)Libermann-Burchard Test	+	+	-	-	-	-
		b)Salkowski reaction	+	-	-	-	-	-
8	Glycoside	General test	-	-	-	-	-	-
9	Cardiac Glycosides	Legal test	-	-	-	-	-	-
		b)Keller-Killiani test	-	-	-	-	-	-
10	Anthroquinone glycoside	Borntrager's test	-	-	-	-	-	-
11	Cyanogenetic glycoside	a)sodium picrate test	-	-	-	-	-	-
		b)mercurous nitate test	-	-	-	-	-	-
12	Coumarin glycoside	a)No aromatic odor	-	-	-	-	-	-
		b)No green fluorescence	-	-	-	-	-	-
13	Saponins	a)Foam test	-	+	-	+	-	+
		b)Lead acetate test	-	-	-	-	-	-
14	Flavonoids	a)Shinoda test	-	-	-	-	-	-
		b)Lead acetate test	-	+	+	-	+	-
		c)Alkaline reagent test	-	-	-	-	-	-
15	Alkaloids	a)Dragendorff's test	-	-	-	-	-	-
		b)Mayer's test	-	+	+	-	+	-
		c)Wagner's test	+	+	+	+	+	+
16	Tannins	Lead acetate test	-	-	+	-	+	-
17	Mucilage		-	-	-	-	-	-
18	Phenol	a)$Fecl_3$ test	+	+	+	+	+	+
		b)Folin-ciocaltaue reagent	+	+	+	+	+	+
19	Starch	Iodine test	-	+	-	+	-	+
20	Organic acids	Calcium Chloride test	-	-	-	-	-	-
21	Volatile Oil		-		-		-	

ME: Methanolic extract; WE: Water extract; (+): Present; (-): Not present.

Table 5: Quantitative Phytochemical analysis

Sr. No.	Phyto-constituents	Mucuna pruriens (L.) DC. (µg/ml)	Mucuna cochinchinensis (Lour.) A. Chev. (White Seed) (µg/ml)	Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed) (µg/ml)
1	Carbohydrates	57.4	146.6	275.8
2	Flavonoids	42.18	35.6	65.0
3	Tannins	203.6	306.2	576.7
4	Glycosides	56.28	10.16	32.8
5	Proteins	110.41	314	270.27
6	Total Phenolic content	10.42	80.68	40.00
7	Lipids	39.2	9.12	10.41

Fig. 1: Graphical representation of quantitation of Carbohydrates

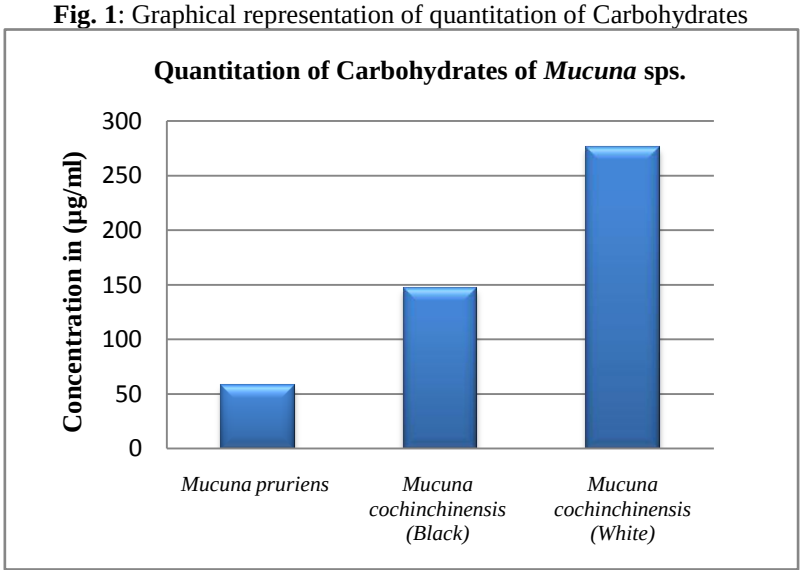


Fig. 2: Graphical representation of quantitation of Flavonoids

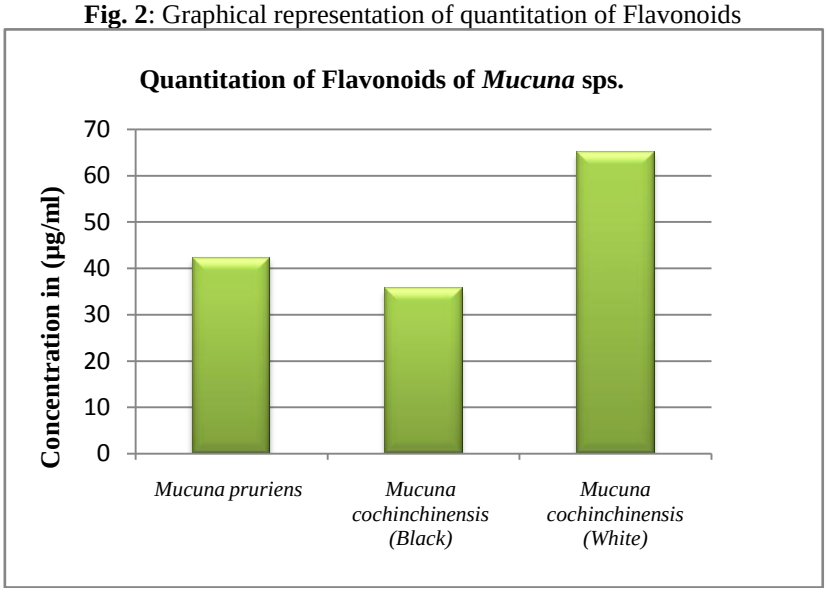


Fig. 3: Graphical representation of quantitation of Tannins

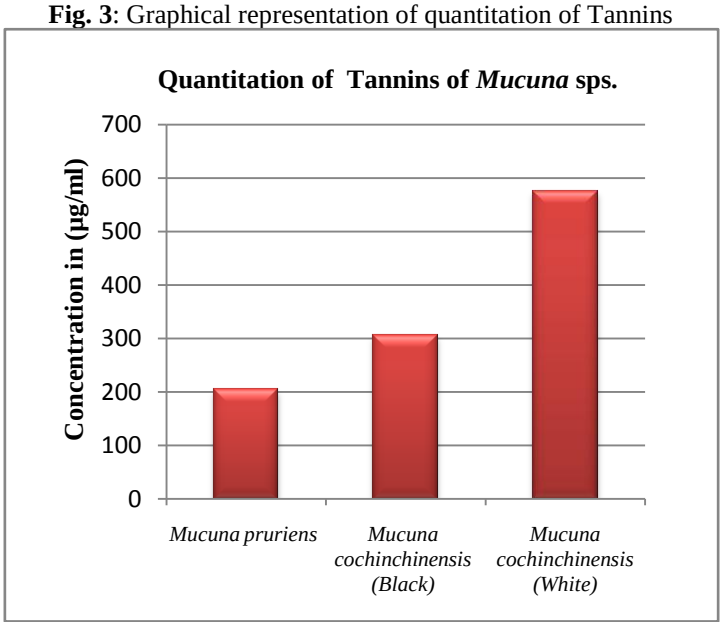


Fig. 4: Graphical representation of quantitation of Lipids

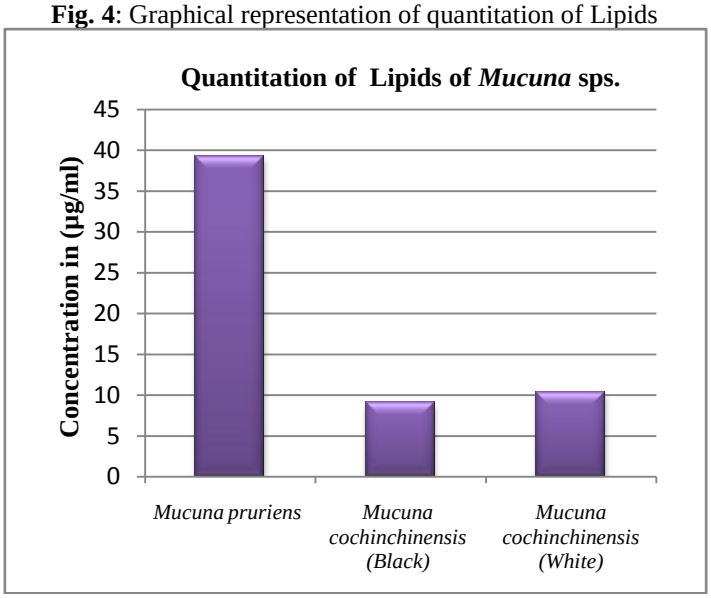


Fig. 5: Graphical representation of quantitation of Proteins

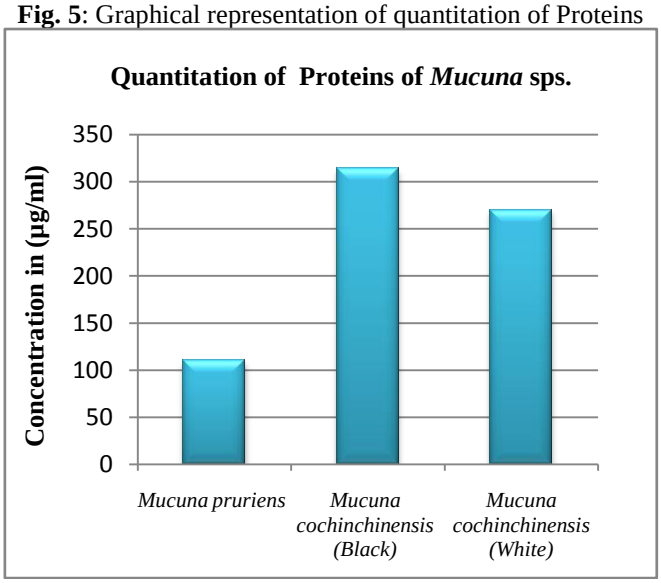


Fig. 6: Graphical representation of quantitation of Phenolic content

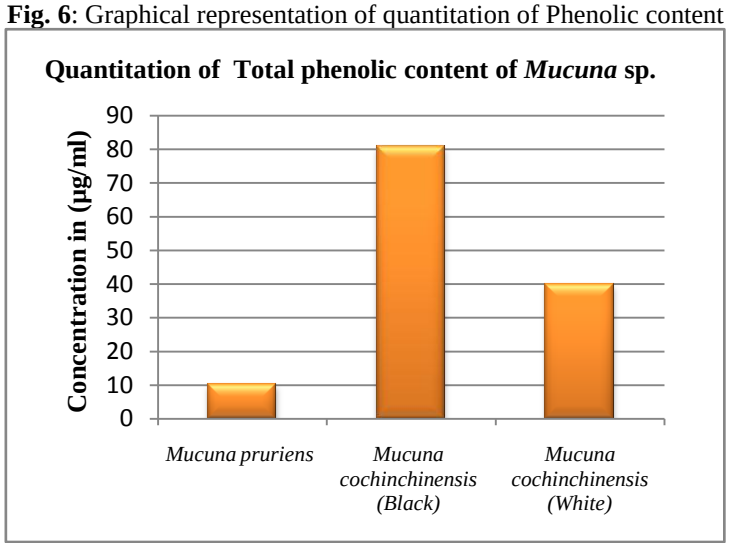


Fig. 7: Graphical representation of quantitation of Glycosides

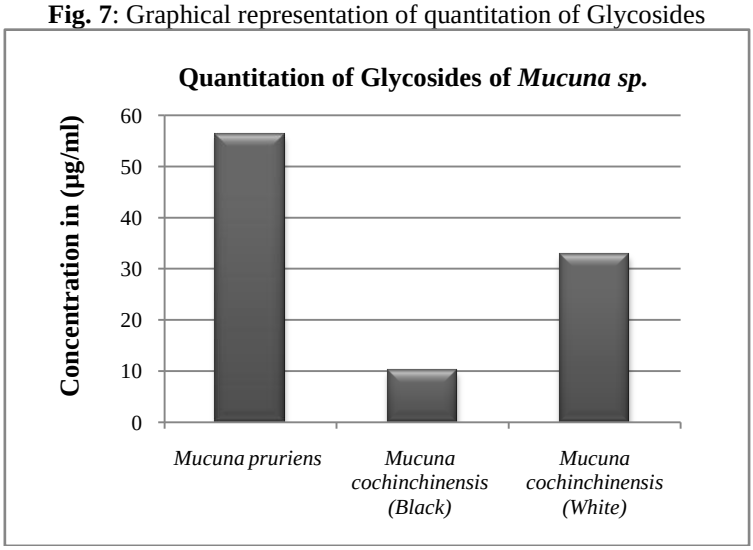


Table 6: Fluorescence of Mucuna species with different solvents

Sr. No.	Solvents	Mucuna pruriens (L.) DC.			Mucuna cochinchinensis (Lour.) A. Chev. (White Seed)			Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed)		
		Day light	Short UV	Long UV	Day light	Short UV	Long UV	Day light	Short UV	Long UV
1	Water	Light brown	Light umber	Light purple	Dark brown	Dark blackish green	Dark purple	Light brown	Chestnut	Dark purple
2	Acetone	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless
3	Benzene	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless
4	Chloroform	Colourless	Colourless	Colourless	Light yellow	Colourless	Colourless	Colourless	Buff	Colourless
5	Carbon tetra chloride	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Very Light brown	Light yellow	Colourless
6	Ethanol	Light yellow	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless
7	Ethyl acetate	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless
8	Methanol	Light yellow	Colourless	Colourless	Light pale yellow	Light pale yellow	Colourless	Light yellow	Light yellow	Colourless
9	Petroleum ether	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless

V. Conclusion

Phytochemical investigation of seed extracts showed the presence of alkaloids, phenols, carbohydrates, reducing sugar, saponins, flavanoids, starch, proteins and hexose sugar at least in one of the extract. These results are supportive with other studies perfomed with Mucuna sps, where same secondary metabolites were found [42]. Qualitative phytochemical screening is an essential step towards discovery of new drug as it provides the information regarding the presence of primary and secondary metabolites in the plant extract. The methods employed to isolate active substance are termed as extractive method. Crude extracts obtained from such extraction can be qualitatively tested to ascertain the presence of different types of components.

Volatile oil, organic acids, mucilage, glycosides, pentose sugar and cystine amino acid was not positive in any of the extract and Mucuna sps under study, indicating the absence of respective phytochemicals whereas, steroid was found to be present only in M. pruriens in less quantity (as the reactions were not very prominent) suggesting that the plant does not possess those phytochemical in abundance. Qualitative tests are used to detect the presence of functional groups, which play very important role in the expression of biological activity. These tests confirm the presence of certain types of phyto-constituents in the sample.

Correlation between the phyto-constituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic disease [43]. Owing to the significance in the above context, such preliminary phytochemical screening of plants is the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy. Antitumor, anti inflammatory and antimicrobial properties are due to the presence of alkaloids [44]. Antiparasitic, antibacterial and antifungal activities are due to the presence of flavonoids [45, 46]. Tannins are used as tanning agents as they possess astringent, antioxidant and antimicrobial activities. Bactericidal and fungicidal properties are due to the presence of tannins [47-48].

In fluorescence the fluorescent light is always of greater wavelength than the exciting light. Light rich in short wavelengths is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substances which do not visibly fluoresce in daylight [49, 50].

The research work was carried out on Mucuna sps. seeds, which showed the presence of various phytochemical constituents and bioactive compounds in all the extracts in varying range and quantity. The quality and quantity of phytochemicals is based on the selection of solvent system and procedure selected for extraction. The selected plant is the source of secondary metabolites. The phytochemical constituents of Mucuna.pruriens and Mucuna cochinchinensis would be helpful in treating many diseases and the fluorescent analysis of powdered drug can play a remarkable role in the determination of quality and purity of the raw drug. These types of studies have vital role because of the commercial and research interest.

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References

[1]. Standley, P.C. and Steyermark, J.A. (1946). Flora of Guatemala. Fieldian: Botany Vol 24 Part V.
[2]. Ding, Y., Kinjo, J., Yang, C. and Nohara, T. (1991). Triterpenes from Mucuna birdwoddiana. Phytochemistry 30(11): 3703-3707.
[3]. Eilittä, M., Bressani, R., Carew, L.B., Carsky, R.J., Flores, M., Gilbert, R., Huyck, L., St-Laurent, L. and Szabo, N.J. (2002). Mucuna as a food and feed crop: an overview. In: Mucuna as a Food and Feed: Current Uses and the Way Forward. Ed. By Flores M, Eilittä M, Myhrman R, Carew L, Carsky R, Workshop held April 26-29, 2000 in Tegucigalpa, Honduras. CIDICCO Honduras. pp. 18-46.
[4]. Janardhanan K. and Lakshmanan K.K. (1985). Studies on the pulse, Mucuna utilis. Chemical composition and anti-nutritional factors. J. Food Sci. Tech. 22: 369-37.
[5]. Mohan, V.R. and Janardhanan, K. (1993). Studies on the Indian tribal pulses. In proceedings of the national seminar on biodiversity: strategies for conservation and future challenges. Department of Botany. Bharathiar University. Ed. by Udaiyan K, Janardhanan K, Manian S, Reddy VRKP. pp. 116-125.
[6]. Manyam, B.V. (1995). An alternative medicine treatment for Parkinson's disease: results of a multicenter clinical trial – HP-200 in Parkinson's disease study group. J. Alternat. Comp. Med. **1**: 249-255.
[7]. Sastry, C.S.T. and Kavathekar, Y.Y. (1990). Plants for reclamation of wastelands. Publications and Information Directorate, New Delhi. p.317-318.
[8]. Singh, U., Wadhwani, A.M. and Johri, B.M. (1996). Dictionary of economic plants in India. Indian Council of Agricultural Research, New Delhi. p. 45-46.
[9]. Wilmot Dear C.M. (1987). A revision of Mucuna (Leguminosae - Phaseolae) in the Indian Subcontinent and Burma. Kew Bull., **42(1)**: 23–46.
[10]. Daxenbichler, M.E., Van Etten, C.H., Hallman, E.A., Earle, F.R. and Barclay, A.S. (1971). Seeds as sources of L-DOPA. J. Med. Chem. **14**: 463.
[11]. British Pharmacopoeia. (1973), London.
[12]. Longhi, J.G., Perez, E., Jose de Lima, J. and Candido L.M.B. (2011). In vitro evaluation of Mucuna pruriens (L.) DC. antioxidant activity. Brazilian Journal of Pharmaceutical Sciences, **47(3)**: 535-544.
[13]. Anonymous. (2005). The Wealth of India Raw Materials, NISCAIR Publishing New Delhi, L-M; **VI**: 439-444.
[14]. Anonymous. (2006). The Wealth of India- Raw Materials. First Suppl Ser. NISCAIR; 166-167.

[15]. Anonymous. Quality Control Methods for Medicinal Plants Materials. Geneva: World Health Organization: 1998, pp. 6-22.
[16]. Khandelwal KR. Practical Pharmacognosy Technique and Experimental. Pune: Nirali Prakashan; 2001, pp. 149-156.
[17]. Subramaniam P, Sunil Kumar. Physicochemical profile of some colouring plants used in Homeopathy. Ancient Science of life, 2001; 21(2): 1-8.
[18]. Harborne JB. Phytochemical methods. Chapman and Hall, London. 1973
[19]. Krishnadevi S, Theymoli B and Sadasivam S. Phenol Sulphuric acid method. Food Chem. 1984. 15: 229
[20]. Sadasivam S, Manickam A. Biochemical methods. New age International (P) Limited, Publishers, New Delhi, 1996.
[21]. Kokate CK. Practical Pharmacognosy. 4th ed. Reprint. Delhi: Vallabh Prakashan; 1996, pp. 10-321.
[22]. Wall ME, Krider MM, Krewson CF, Eddy CR, Willaman JJ, Correll DS, Gentry HS. Steroidal sapogenins – Survey of plants for steroidal sapogenins and othe constituents VII, Jour. Amer. Pharm. Ass., Sci Ed., 1954, 43: 1-7.
[23]. Trease GE, Evans WC. Pharmacognosy. 11th edn; Brailliar Tiridel Can. Macmillian Publishers 1989.
[24]. Harborne JB. Phytochemical Methods—A Guide to Modern Techniques of Plant Analysis. 1stedn: Chapman and Hall; London, UK. 1998.
[25]. Harbourne JB. Phytochemical Methods: A Guide to Modern Technique of Plant Analysis, 2nd ed. Chapman and Hall, London. 1984; 282.
[26]. Sofowora A. Phytochemical screening of medicinal plants and traditional medicine in Africa 2nd Edition Spectrum Books Limited, Nigeria, 1993, 150-156.
[27]. El-Tawil BDH. Chemical constituents of indigenous plants used in Native Medicines of Saudi Arabia, II. Arab Gulf J Sci Res, 1983; (12): 395 -410
[28]. Siddiqui AA, Ali M. Practical Pharmaceutical Chemistry. 1st edn; CBS Publishers and distributors, New Delhi, 1997; 126-131.
[29]. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. Internationale Pharmaceutica Sciencia, 2011; 1: 98-106
[30]. Brain KR, Turner TD. The practical evaluation of phytochemicals. Wright Science Technical:Bristol: 1975
[31]. Kokate CK. A Textbook for Practical Pharmacognosy. 5th Ed edn. 2005.
[32]. Gibbs RD. Chemotaxonomy of Flowering Plants. Vol.1, McGill Queen's University Press Montreal and London. 1974.
[33]. Peach K., Tracey MV. Modern methods of plant analysis. Vol.3, Springer Verlag, Berlin. 1956.
[34]. Khatiwora E, Adsul VB, Kulkarni MK, Deshpande NR, Kashalkar RV. Spectroscopic determination of total phenol and flavonoid contents of Ipomoea carnea. International Journal of ChemTech Research, 2010; 2(3), 1698-1701.
[35]. Trease GE and Evans WC. Pharmacognosy. 17th edn; Bahive Tinal, London. 1985, PP 149.
[36]. Lowry OH, Rosebrough NR, Lewis Farr A, Randall RJ. Protein measurement with the Folin Phenol Reagent, J. Biol. Chem, 1951; 193: 265-275.
[37]. Ganai BA, Masood A and Zargar MA. Experimental Biochemistry, 1st ed., Valley Publications, J&K, 2005; 148-149.
[38]. Mallick CP, Singh MB. Plant enzymology and Histoenzymology. Kalyani publishers, New Delhi 1980, 286.
[39]. Nanna RS, Banala M, Pamulaparthi A, Kurra A, Kagithoju S. Evaluation of Phytochemicals and Fluorescent Analysis of Seed and Leaf Extracts of Cajanus cajan L. Int. J. Pharm. Sci. Rev. Res., 2013; 22(1): 11-18.
[40]. Birajdar VV, Mhase AG, Gurav AM, Murthy SN. Preliminary pharmacognostic and phytochemical standardization of Dhataki [Woodfordia fruticosa (L.) Kurz.] leaves. Ayu 2014; 35:309-15.
[41]. Chase CR, Pratt RJ. Fluorescence of powdered vegetables drugs. Indian Journal of Experimental Biology, 1949; 33(6): 428-432.
[42]. Manalisha D and Chandra KJ. 2012. Priliminary phytochemical analysis and acute oral toxicity study of Mucuna pruriens L. in Albino mice. International Research journal of Pharmacy, 3(2): 181-183.
[43]. Pandey P, Mehta R, Upadhyay R. Physico-chemical and preliminary phytochemical screening of Psoralea corylifolia. Arch Appl Sci Res, 2013; 5:261-265.
[44]. Castilhos TS, Giordani RB, Henriques AT, Menezes FS, Zuanazzi JA. In vitro evaluation of the antioxidant, anti-inflammatory and antimicrobial activities of the montanine alkaloid. Braz J Pharmacogn, 2007; 17: 209–214.
[45]. Simões CM, Schenkel EP, Gosmann G, Mello JC, Mentz LA, Petrovick PR, Porto Alegre/ Florianópolis, Ed. Universidade/ UFRGS/ Ed. da UFSC, 4, Farmacognosia: Da planta ao medicamento. 2002.
[46]. Sen G, Mandal S, Saha Roy S, Mukhopadhyay S, Biswas T. Therapeutic use of quercetine in the control of infection and anemia associated with visceral leishmaniasis, Free Radic Biol Med, 2005; 38: 1257–1264.
[47]. Ho KY, Tsai CC, Huang JS, Chen CP, Lin TC, Lin CC. Antimicrobial activity of tannin components from Vaccinium viti-sidaea L. J Pharm Pharmacol, 2001; 53: 187– 191.
[48]. Killedar SG, More HN, Estimation of tannins in different parts of Memecylon umbellatum Burm, J Phar Res, 2010; 3(3): 554-556.
[49]. Arya V, Gupta R, Gupta VK. Journal of Chemical and Pharmaceutical Research, 2011; 3(3), 447-456
[50]. Kumar D, Kumar K, Kumar S, Kumar T, Kumar A, Prakash O. Asian Pacific Journal of Tropical Biomedicine, 2012; 169-175.

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THE VELVET BEAN (*MUCUNA SPS.*): IN AYURVEDIC ERA

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ABSTRACT

Velvet Bean, is an important '*Vajikarana'* drug mentioned in Ayurveda, an integral part of Indian System of Medicine. It is used mainly for its *Balya*, *Vrishya* and *Tridoshahara* properties. It is the best natural source of L-DOPA, which is precursor to the neurotransmitter dopamine, widely used in Parkinsonism, muscular disorders and also in spermatogenesis. This review has highlighted the importance of *Kapikacchu* in various *Samhitas* and *Nighantus*. The review summarized the classical names, classification, properties, uses of drug and its *Yogas* (formulations). The literature survey showed that in India *Kapikacchu* species are widely used to treat the patients of various disorders since ancient times. This review evaluates the rich heritage of traditional drug, "*Kapikacchu*".

KEYWORDS: *Kapikacchu, Nighantu, Samhita, Rasapanchaka, Vajikarana.*

INTRODUCTION

Ayurveda is a comprehensive scientific system of medicine evolved in India. Initially, it was developed through ancient wisdom, clinical experiences and experimentation in scientific, technological and medical parameters. Ayurveda means "The Knowledge of Life". In Sanskrit, Ayurveda word consists of '*Ayur*' for life and '*Veda*' for science or knowledge. Ayurveda, ancient science of life believes in the philosophy of not only treating the diseases but also to help in maintaining the healthy state of every individual.

The Hindu philosophy of medical science is mainly based on four *Vedas viz.* the *Rig Veda* (3000 – 2500 BC), *YajurVeda* (1200 to 1000 BC), *SamVeda* (1200 to 1000 BC) and *Atharva*

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Veda (1200 – 1000 BC). Ayurveda is based on the vedic philosophy of healthy living of the person which is prescribed in the *Atharva veda*. So it is considered to be the *Upaveda* (supplementary subject) of the *Atharva Veda*. The *Atharva Veda* has more descriptive details of large number of medicinal plants used in many diseases as compared to other *Vedas* due to addition of the knowledge by advance of time.

Current knowledge about Ayurveda is primarily based on *Brihattrayi* and *Laghutrayi*. *Brahatrayi* includes *Caraka Samhita* by Caraka; *Sushruta samhita* by Sushruta; *Ashtanga Hridaya* and *Ashtanga Sangraha* by Vagbhata. *Laghutrayi* includes *Sharangadhar Samhita* by Sharangadhara; *Bhavprakash Nighantu* by Bhavmishra; *Madhavnidan* by Madhavakara.

The word *Nighantu* is derived from the term "*NIGAMA*" means which brings out the extremely concealed or secrete meaning of words in systemic way. *Nigama, Nirukta,* and *Nighantu* are used as synonyms. *Rajanighantu* declares a physician without the knowledge of *Nighnatu* is like scholar without the knowledge of vyakarana (grammar) and a soldier without weapons. This explains the knowledge of *Nighantus* is indispensible for a physician.

Kapikachu, is a one of the well known *Vrishya* (Spermatogenetic activity) drug mentioned in Ayurvedic texts right from *Caraka* and *Sushruta samhita* period. It is also used as a drug for *balya* (promotes muscle mass and body weight) and advocated to use for *vajikarana chikitsa* (Aphrodisiac). Every part of the plant have medicinal value. Seeds and roots are mentioned as most important parts, which are good source of giving vital energies. According to the traditional and scientific data available, legumes have promising potential source in terms of nutrition, medicine and agricultural development in developing countries.

The roots are bitter, sweet, thermogenic, emollient, stimulant, purgative, aphrodisiac, diuretic, emmenagogue, anthelmintic, febrifuge and tonic. They are useful in vitiated conditions of *vata* and *pitta*, constipation, nephropathy, strangury, dysmenorrhoea, amenorrhoea, elephantiasis, dropsy, neuropathy, ulcers, helminthiasis, fever, cholera and delirium. The leaves are aphrodisiac, anthelmintic, tonic and are useful in ulcers, inflammation, helminthiasis, cephalagia and general debility. Seeds are astringent, laxative, anthelmintic, aphrodisiac, alexipharmic and tonic. They are useful in gonorrhea, consumption, sterility and general debility. The pods are used as anthelmintic. The hairs of legume and flowers are vermifuge to expel ascarids.[1-4] The roots are useful in hemiparesis and facial palsy. Seed powder is useful in parkinson disease.[5] Seeds are useful in *Apabahuka* (Frozen shoulder) to

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increase the strength of arm and also as aphrodisiac. An ointment prepared with pod hair acts externally as a local stimulant and mild vesical.[6] Pod hairs are useful in *krimi*.[7,8] In addition, *Mucuna* is also traditionally used in various other applications like, dye.[9] Treatment of pain and numbness of joints and irregular menstruation.[10]

Seeds are excellent source of L-DOPA which is precursor of dopamine a neurotransmitter used in the treatment of Parkinson's disease.[11,12] Almost all the species are reported to contain L-3,4-dihydroxy phenylalanine (L-DOPA), a non-protein amino acid which acts as a neurotransmitter.[13]

Tan *et al.* (2009); Fung *et al*., (2012) revealed the antivenom activity of seeds.[14,15] Vadivel and Janardhan (2000) investigation suggested that the antidiabetic activity may be due to its dietary fiber content.[16] Bhaskar *et al.*, (2008) study showed that *Mucuna pruriens* could be a source of hypoglycemic compounds.[17] *M. pruriens* not only reactivates the antioxidant defence system of infertile men but also helps in the management of stress and improves semen quality.[18] Clinical study of "*Kapikacchu Churna*" was effective to increase the sperm count.[19,20] Sperm count and motility were significantly recovered in infertile men.[21] The extract of whole plant had antimicrobial properties against both Gram positive and Gram negative organisms and also showed antitumor and antioxidant effects.[22] Hussian and Manyam, (1997) revealed that *M. pruriens* showed twice the anti-Parkinsonian activity than synthetic L-DOPA.[23] Similarly, seed powder preparation was effective in Parkinson disease patient than standard drug *viz.* Levodopa or Carbidopa.[24] Nicotine adenine dinucleotide (NADH) and coenzyme Q-10 were present in the *M. pruriens* cotyledon powder which shows the effect in Parkinson's disease.[25] Patil *et al.*, 2010 study revealed the anti depressant activity.[26] Seed also showed the anti-inflammatory activity.[27,28]

In Vedic literature, Kapikachu is not mentioned. Even in *Brihattrayi* the term *Kapikachu* is not widely used. All 3 *Samhitas* (Caraka, Sushruta, Vagbhata) described this plant majorly with the terms like *Atma gupta*, *Svayam gupta* and also *Markati* (C.S.Ci. 9/52; S.S.Ut. 29/7 & 33/7; A.H. Ut. 6/34).

Classification of Kapikachu

Samhitas and *Nighantus* classified a Kapikachu in different *Ganas* or *Varga* as follows (Table 1).

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Table 1: Classification of Kapikachu

S.No.	Texts	Gana / Varga	References
1.	Caraka Samhita[29]	Balya, Madhura Skanda, Purisa Viranjaniya	Ca.Su-4/7, Ca.Vi-8/139, Ca.Su-4/32
2.	Sushruta Samhita[30]	Vidarigandhadi, Mudgadivarga, Kakolyadi, Vata samsamana	Su.Su- 38/4, Su.Su- 46/36, Su.Su- 37/26
3.	Ashtanga Sangraha[31]	Vidaryadi, Durvadi,	A.S. Su-16/2
4.	Ashtanga Hridaya[32]	Vidaryadi	A.H. Su-15/9
5.	Ashtanga Nighantu[33]	Vidaryadi	A.N. 13
6.	Dhanvantari Nighantu[34]	Guducyadi	D.Ni.46/159
7.	Kaiyadeva Nighantu[35]	Ausadhi	K.Ni.154/11
8.	Bhavaprakasa Nighantu[36]	Guducyadi	B.P.Ni. 57/131
9.	Raja Nighantu[37]	Guducyadi	R.Ni.50/52
10.	Sodhal Nighantu[38]	Guducyadi, Laxmanadi	S.Ni. 171-172 / ; 199-200 S.Ni. 648
11.	Madanpal Nighantu[39]	Abhayadi	M.Ni.271-
12.	Priya Nighantu[40]	Pipalyadi	P.Ni.21/48-50
13.	Nighantu Adarsh[41]	Palashadi	Pg 453

Classical names of Kapikachu and their meaning

The authors of different Ayurvedic texts have mentioned several classical names to the medicinal plants and minerals, while explaining their morphology and properties. In the same way for *Kapikachu* also different classical names were given by scholars (Table 2).[41]

Table 2: **Classical names of Kapikachu and their meaning**

Classical Names	Its meaning
Atma gupta	Protects self with the help of its hair.
Ajada	Potent Aphrodisiac
Durabhigraha	The fruits are difficult to handle
Dusparsha	Produces irritable sensation on touching the fruit
Markati	Hairy like monkeys tail
Kacchura	Causes itching
Kandura	The hairs produce intense itching
Kapikachu	Causes itching to monkeys also
Kapiromphala	Pod hair like that of monkey's hair
Languli	Legume shape is like that of monkey's tail
Pravrishayana	Climber growing in rainy season
Suka simbhi	Pods with hairs
Svayamgupta	The plant is protected by the hairs on fruits
Vanshukari	Climber grows wildly
Vrishya	Acts as an aphrodisiac

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Vrushabhi	A potent aphrodisiac
Vrushabeeja	The seeds are potent aphrodisiac
Vyanga	Fruits are curved

Rasa Panchaka of Kapikachu

According to Ayurveda the knowledge of *Rasa panchaka*, that is *Rasa, Guna, Virya, Vipaka, Prabhava* are very essential to understand the activity and potency of any drug. *Guna* and *Karma* are Ayurvedic pharmacodynamics of the plant. *Rasapanchaka* and *dosha karma* of *kapikacchu* mentioned by different *Nighantus* are given below in Table 3.

Table 3– Rasa Panchaka of Kapikachu

Rasa Pancaka		References							
		D.N.	M.N.	K.N.	B.N	R.N	S.N	P.N.	API
RASA	Madhura	+	+	+	+	+		+	+
	Tikta	+		+	+		+	+	+
GUNA	Guru		+	+	+	+	+	+	+
	Snigdha				+		+		+
VIRYA	Sita	+		+		+			+
VIPAKA	Madhura						+		+
DOSHA KARMA	Vatahara	+	+	+	+	+	+	+	+
	Kaphahara				+				+
	Pittahara	+	+	+	+	+	+		+

+ present

(D.N. – Dhanvantari Nighantu[34]*; M.N. – Madanapala Nighantu*[39]*; K.N. – Kaideva Nighantu*[35]*; B.N. – Bhavaprakash Nighantu*[36]*; R.N. – Rajanighantu*[37]*; S.N. – Sodala Nighantu*[38]*; P.N. – Priya Nighantu*[40]*; API – Ayurvedic Pharmacopoeia of India* [42]*)*

Prayojyanga of Kapikachu (Useful part)

Seed, Root, Pod Hairs, Leaves, Flower.[2,3,8]

KAPIKACHU IN SAMHITAS

Caraka samhita (1000 BC – 4 AD)[29,43]

Among the existing old medical texts of India, *Caraka samhita* is considered as the oldest one. This *samhita* was composed by Agnivesa (1000 BC), redacted by Caraka (2nd BC) and reconstructed by Dridhabala (4th AD). Caraka samhita is mainly focused on the medical management of diseases that is *Kayachikitsa,* branch of Ayurveda. Acharya Caraka has mentioned *Kapikachu* with the name of *Rishabhi* in *balya dasaimani varga*; as *Kachura* in *PurisaViranjaniya dasaimani varga* and as *Rishyaprokta* in *madhura skanda dasaimani varga kashayas*. He also used the term *Adhyanda* (C.S.Chi.3/266) for Kapikachu. Caraka

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mentioned different *yogas* (formulations) of Kapikachhu to treat the diseases such as Bramha rasayana in *Medho vridhi* (Obesity) (C.S.Chi.1/1-58); Vrisya pupalika for *Vrishya* (spermatogenetic activity) (C.S.Chi.2/2; 2/3-15); Maha paisachika Ghrita indicated in Fever, Insanity, Seizures (C.S.Chi.9/45-48); Swadamstradi Ghrita in Vata Pitta hara diseases (C.S.Chi.11/44-47); Tryusanadya Ghrita used in *Kasa* (cough), Jwara, Gulmahara (C.S.Chi.18/39-42); Medicated Ghrita for Svasa, Kasa, Grahani (C.S.Chi.26/80-82); Jivantyadi Anuvasana Yamakam indicated in Brimhana,Vata pitta nasaka, Balya, Sukra Agnivardhaka (C.S.Si.4/9-11).

Sushruta Samhita (1000 BC – 4th AD)[30,44]

This Samhita is composed by Vridha Sushruta which is chiefly dealt with different aspects of *Shalya chikitsa* (surgical and parasurgical mode of treatment) in Ayurveda. It again redacted by Sushruta in 2ndAD. Later, probably revised by Acharya Nagarjuna, the greatest scientist in 5thAD. In the existing form, it appears to be much later than Caraka samhita, because its paatha sudhi was done by Chakradata in 10th century. *Kapikachu* is mentioned in *Vidarigandhadi* and *Vata samsamana ganas*. Susruta has used Kisimbivalli (S.S.Su 46/46) and Languli (S.S.Su 19/29) as synonyms for Kapikachu. Properties of Kapikachu beeja were mentioned separately for the first time in this Samhita (S.S.Su 46/36). Preparations and indications in which Kapikachu are mentioned such as Vrishya yoga for Vrishya action (S.S.Chi26/30-35); Jeevantyadi Anuvaasana Yamakam indicated in Vrishya, Deepana (S.S.Chi37/23-26); Kapikachu churna used in Pitta, kapha vidaghda drishti diseases (S.S.U17/7); Kapikachu Madhuyashti yoga for Vamana hara (S.S.U49/29); Kapikachu Ikshurasa Pippali Ghrita yoga in *Mutraghata* (urinary obstruction), *Vandhyatwa* (infertility) (S.S.U58/53-57).

Ashtanga Sangraha[31]

This Samhita is composed by Vridda Vagbhata in 6th AD. Kapikachu is mentioned in *Durvadi* and *Vidaryadi ganas* (A.S.Su-16/2). Vagbhata mentioned different *yogas* of Kapikachhu in various diseases such as Kapikachu sidda Ghrita used for Nasal drops to *Garbhini* (pregnant) (A.S.Su.29/11 and A.S.Su.7/31); Kapikachu mardana for *Sannyasa* (total loss of consciousness) and *Madatyaya* (condition caused by the excessive use of alcohol) (A.S.Chi9/125); Nagabala ghrita in Kshata kshaya, Pitta rakta diseases (A.S.Chi5/69; A.S.Chi 5/53); Amrita taila for Vata vyadhi chikitsa, Agnideepana (A.S.Chi.23/72; A.S.Chi23/86); Kashaya preparation in *Pittaja Chardi* (vomiting) (A.S.Chi.8/19); Raja Yapana Basti and

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Yapana Basti for Vrishya (Aphrodisiac) activity (A.S.Ka.5/23; A.S.Ka5/34); Medicated Oil for Abhyanga, *Paalitya Chikitsa* (premature greying of hair) and Vata Pitta diseases related to Shukra and Rajas (A.S.U.1/80, A.S.U.28/46; A.S.Ka.5/49); Maha kalyana Ghrita for Sannipaata vyadhis, Sthoulya (Obesity) (A.S.U.9/30); Maha paishachaka ghrita in Unmada, Apasmara, Sannipata Jwara (A.S.U.9/32); Maha mayura Ghrita cures different Indriya vyadhis, Sirorogas (A.S.U.28/73); Saradi Ghrita and Medicated Ghrita in Vrishya Chikitsa (A.S.U.50/26,30; A.S.U.50/62); *Ksheera* (milk) preparation with Beeja churna used in Vrishya, Visha (A.S.U.48/57; A.S.U.50/39,47); Kapikachu mulasidda Ksheera and Ghee for Vrishya (A.S.U.50/64); Svayamgupta Churna Yoga in Vrishya (A.S.U.50/58,60); Kapikachu sidha Poopalika with Ghrita and Padalepa Yoga indicated in Vrishya (A.S.U.50/65, 68,69; A.S.U.50/106, 108).

Ashtanga Hridaya[32,45]

This samhita is written by Laghu Vagbhata during 7th AD. Kapikachu is mentioned in *Vidaryadi Gana* (A.H.Su-15/9) both Vagbhatas have also mentioned different *yogas* of Kapikachu keeping its Vrishya activity in mind. These are Vidaryadi Ghrita in Vrishya (A.H.U.2/21, 31, 1/23); Medicated Ghee for Cough (A.H.Chi 3-38); Amrita prasa ghrita in Cough, Hiccup (A.H.Chi 3/94-101); Svadamshtradi Ghrita in Vata pitta Hridya sula, Dysuria (A.H.Chi.3/102-103). Agastya and Vashishta Rasayana (Hareetaki Rasayana) for Rasayana purpose (A.H.Chi 3/127-132,133); Jeevantyadi Kalpa (Vata pitta hara kalpa) in Asthapana basthi (Retention enema) (A.H.Ka.4/60); Medicated oil Lepana for Karna pali Sandhana (A.H.U.40/17); Beeja churna, masha flour with Ksheera; Kapikachu beeja churna along with svadamshtra, ikshuras, satavari in milk indicated in Vrishya (A.H.U.40/31, 34); Kapikachu used as diet for Vrishya (A.H.Su.6/22).

Laghutrayi and Chikitsa grantha

Like in Samhitas, Kapikachu is also mentioned in Chikitsa granthas as a synonyms *viz Atmagupta, Kandura* etc. and in the management of several diseases.

Chakra Datta (Chakrapani in 11th Cent A.D.)[46]

Kapikachu is mentioned for different diseases apart from its vajikarana activity along with other drugs. For Vatavyadi Balamuladi Nasya, Chatushtayam Mashadi Kashaya (Maha Baladi kwath) (C.D.22-27, 28) were used. Svaguptadi leha in Mutraghata (C.D.33-17,18); Svayamguptadi churna, Gokshuraadi churna and Godhumadiya ghrita used for Vrishya (C.D.67/6; 66/26-34; 67/26-36).

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Vangasena Samhita[47]

Kapikachu is mentioned in Vajikarana activity along with other drugs such as Brihad Aswagandhadhi ghrita (V.S.84/22-27); Medicated Ghrita (V.S.84/65-66); Gokshura Kapikachu beeja sidha ksheera (V.S.84/73); Masha Ghrita (V.S.84/75-79); Godhumaadi Ghrita (V.S.84/80-89) and Jeevanti Yamaka indicated in Anuvasana, Nasya, Panaartha, *Urdhva jatrugata roga* (diseases of head and neck, throat) (V.S.84/93-95).

Yoga Ratnakar[48]

In this text, Kapikachu is described in Uttarakahanda, Vaajikarana adhyaya. Preparations and indications in which Kapikachu is mentioned for Vajikarana purpose like Vanari gutika (Y.R.Ut.11/27-31); Kesar paka (Y.R.Ut.11/49-56); Rati modaka (Y.R.Ut.11/57-60); Kameswara modaka (Y.R.Ut.11/68); Kameswar samdi paka modaka (Y.R.Ut.11/77-83). Madana kamadeva rasa (Y.R. Ut. 11/117); Rativallabha Pugapaka (Y.R. Ut. 11/61,67); Satavaryadichurna (Y.R.Ut. 11/146); Kapikachu paka (Y.R.Ut. 11/156-160).

Bhaishajya Ratnavali (Govinda Das In 18th cent A.D.)[49]

Kapikachu preparations along with other drugs such as Gokshuradi yoga (B.R. 70/15); Godhumadya ghritam (B.R.70/18); Brihadaswagandha ghritam (B.R.70/19); Bricchatavari modaka (B.R.70/21-24); Rativallabha modaka (B.R.70/25); Mahakameswara modaka (B.Ra.70/26); Kamagni sandipana modaka (B.R70/27); Manmadhabhra rasa (B.R70/29) were used in vajikarana activity. Preparation of Svayam gupta, Ikshuraka yoga used in *Dhaatukshaya* (depletion of body tissues) (B.R.70/14). Preparation like Yavana krit yoga (Mophara-Mopharava) was consumed by Kings for *vrishya* purpose and also in all diseases (B.R.70/30).

Gada Nigraha[50]

Gada Nigraha mentioned different yogas (formulations) of Kapikachhu for Vaajikaranartha such as Mashadi Ghritam (G.Ni.9-12); Vajikaropayas Yoga (G.Ni.14); Vajikaram Masha parnyadi churnam (G.Ni.16); Vajikaram Svayamguptadi churnam (G.Ni.19; G.Ni.29); Vajikaram Svayamguptadi Modakam (G.Ni.31-35,37); Vajikaram Sharmuladi Yoga (G.Ni.40-42); Vajikari Godhumadi Pupalika (G.Ni.43-44); Vajikaram Ikshurkadi Churnam (G.Ni.49).

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Harita Samhita[51]

Harita Samhita mentioned preparations (formulations) of Kapikachhu like Visadi churna (H.Sa.47/9); Guduchyadi Churna (H.Sa.47/13,14) for Veerya vardhaka and Vajikaran purpose.

KAPIKACHU IN NIGHANTUS

Nighntus are just like Ayurvedic pharmacopoeia which explain the characters of medicinal plants in the form of classical name, Ayurvedic properties (Rasa panchaka), uses, actions and formulations.

Ashtanga Nighantu (8th cent A.D.)[33]

In this Nighantu, Acharya Vahata has described maximum group of drugs on the basis of classification of Ashtang Sangraha and Ashtanga Hridaya. Following synonyms are mentioned i.e. Markati, Atmagupta, Kapikachhura, Vrishyabeeja, Kandukari and Shardula vigraha.

Dhanvantari Nighantu (10th -13th cent AD)[34]

Kapikachu is mentioned in *Guduchyadi varga* along with its classical names like Kapikachu, Atma gupta, Svyam gupta, Maharshibhi, Languli, Kandula, Chanda, Markati, Durabhigraha. Kapikachu is having madhura, tikta rasa, sheeta virya, and vata nashaka, vrishya (Aphrodisiac), dushta vrana nasini (indolent ulcers). Atmagupta beeja taila is mentioned with having Madhura, Kashay rasa and Guru Ushna, Snigdha guna.

Sodhala Nighantu (12th cent. AD)[38]

This Nighantu was composed by Sodhala in two parts named as Naamasangraha dealing with synonyms and Guna-Sangraha dealing with properties and actions. Kapikachu is classified in two vargas namely *Guduchyadi* and *Laxamanadi varga*. *Guduchyadi varga* mention the classical names like Atmagupta, Svayamgupta, Ajaharsini, Languli, Kandura, Vyanda, Markati, Durabhigraha, Badari, Kapiroma phala, Dusparsha, Kacchura, Kharju, Romalu, Vanasukari and in Laxmanadi varga, classical names like Laghukacchu, Cara, Malu, Lomasha, Alpakacchuka, Vatavidhvansini and Santativardhini. It was useful in *Balya* and *vrishya* activity. Sodhala also mentioned that *atiyoga* (excess use) increases pitta and causes mada and *vibhrama* (inebrient state).

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Abhidhanaratnamala (Sadrasa Nighantu) (13th cent. AD)[33]

Kapikacchu, described in *Svadu skandha* along with synonyms like Markati, Atmagupta, Kandura, Kapikacchu, Rhishabhi, Jangali, Kandukari, Shardulavighraha, Rhishyaprokta, Mahagupta and kapiromaphala.

Madanapala Nighantu (Madana Vinoda) (14th Cent AD)[39]

Kapikachu is mentioned in *Abhayadi varga*. Madanapala enumerated Kapikachu with its classical names like Svayamgupta, Kandula, Duravagraha, Canda, Atmagupta, Languli, Markati, Praharshini with properties Madhura rasa, Guru guna and actions like Vrishya and Brimhana. Kapikacchu is said as best for Vajikarana.

Kaideva Nighantu (Pathya Pathya Vibhodaka) (15th Cent)[35]

In *Kaideva Nighantu*, Kapikachu is described under classical names such as Rhushyaprokta, Sukasimbhi, Kapiromphala, Rhushabhi, Kapikacchu, Atmagupta, Kandura, Kandukari, Pravrushyani, Ajaha, Gupta, Svayam gupta, Markati, Kapiloma, Vrusya, Adhyanda, Vamani, Durabhigraha and having properties like Madhura, Tikta rasa, Seeta virya, Guru guna, Vata pitta kapha and Rakta dosha nashaka. He has mentioned that, this drug has the properties similar to Masha and possesses *Brimhana* (improves weight), *Vrishya*, Raktapitta hara and Vrana shodhana qualities.

Bhavaprakasha Nighantu (16th Cent A.D.)[36,52]

The period of Bhavamisra is at the junction of the medieval and modern periods and an important landmark in the history of Indian medicine. He classified the Kapikachu in *Guduchyadi varga*, with the synonyms like Ajada, Atmagupta, Dusparsha, Kandura, Languli, Markati, Pravrshayani, Sukasimbi, Vrishya and Vyanga. It posses Madhura, Tikta rasa, Guru guna, with the actions of tridosha samaka, vrishya. He mentioned the Kapikachu beeja was best drug for Vajjikarana (aphrodisiac).

Rajanighantu of Pandit Narahari (17th Cent A.D.)[37]

Kapikachu is mentioned in *Guduchyadi varga* and described this drug with its synonyms like Vaanari, Languli, Atmagupta, Kandura, Markati, Sukasimbi, Svayam gupta, Kundali, Canda, Durabhigraha, Maharshabhi, Karpi, Romphala, Gupta, Dusparsha, Kacchura, Jaya, Pravarushyani, Badari, Guru, Aarshabhi, Shimbhi, Vaarahika, Tikshna, Romalu,Vanshukari, Kisharoma, Romavalli. It is mentioned that this drug possess Madhura rasa, Virya Vardhaka and useful in Vataroga, Kshaya, Shita pitta, Raktapitta and Vrana nashaka.

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Rajavallabha Nighantu (18th Cent AD)[53]

This Nighantu is composed by Rajavallabha Vaidya and redacted by Narayana Dasa. This text deals with dietary articles with their properties and actions. This text is divided into six chapters.

KAPIKACHU IN MODERN ERA

Saligrama nighantu, Nighantu Adarsha and Priya nighantu, Ayurvedic Materia Medica, Wealth of India and other books written by recent Acharyas which provide the information about its habit, habitat, morphology, chemical composition etc.

Saligrama nighantu (19th Cent AD)[54]

He has quoted the kapikachhu in *Guduchyadi varga* with classical names Vaanari, Languli, Adhyanda, Atmagupta, Ajada, Kapiprabha Kandura, Jada, Markati, Sukasimbi, Sukapindi, Svayam gupta, Rhushyaprokta, Sadhyashotha, Suka, Sukvati, Gatrabhanga, Kacchumati, Rhushabhi, Ajahba, Vyaghra Canda, Durabhigraha, Maharshabhi, Kundali, Romphala, Gupta, Dussparsha, Kacchura, Jaya, Pravarushyani, Badari, Guru, Aarshabhi, Shimbhi, Vaarahika, Tikshna, Romalu,Vanshukari, Kashiaroma, Romavalli, Vyanga, Vryshya.

Priya Nighantu (P.V.Sharma)[40]

Priyavrat Sharma mentioned Kapikachu in Pippalyadi varga with the synonyms like Atma gupta, Markati, Shuka simbhi and also mentioned the guna karma of Kapikachu as Madhura, Tikta rasa, Guru guna, with Brunhana, Vata shamaka, Balavardhaka karmas.

YOGAS (FORMULATIONS) OF KAPIKACHU

Kapikachu in different yogas mentioned by various samhitas**.**[29-33, 46-51, 55,56]

Table – 14: Yogas (Formulations) of Kapikachu

S. No.	Name of the preparation	Indication	Reference
1.	Bramha rasayana	Medho vridhi	C.S.Chi1/1-58
2.	Vrishya yoga pupalika	For Vrishya action	C.S.Chi. 2/2; 2/3-15
3.	Maha paisachika Ghrita	Fever, Insanity, Seizures	C.S.Chi9/45-48
4.	Swadamstradi Ghrita	Vata Pitta hara	C.S.Chi.11/44-47
5.	Tryusanadya Ghrita	Kasa, Jwara, Gulmahara	C.S.Chi.18/39-42
6.	Medicated Ghrita	Svasa, Kasa,Grahani	C.S.Chi.26/80-82
7.	Jivantyadi Anuvasana Yamakam	Brimhana, Vatapitta nasaka, Bala, Sukra Agnivardhaka.	C.S.Si4/9-11
8.	Vrishya yoga	Vrishyata	S.S.Chi26/30-35
9.	Jeevantyadi Anuvaasana	Vrishya, Deepana.	S.S.Chi37/23-26

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	Yamakam		
10.	Kapikachu churna	Pitta, kapha vidaghda drishti	S.S.U17/7
11.	Kapikachu Madhuyashti yoga	Vamana hara	S.S.U49/29
12.	Kapikachu,Ikshurasa, Pippali Ghrita yoga	Mutraghata, Vandhyatwa	S.S.U.58/53-57
13.	Kapikachu sidda Ghrita	Nasal drops to Garbhini	A.S.Su.29/11 and A.S.Su.7/31
14.	Kapikachu mardana	Sanyasa/ Madatyaya	A.S.Chi9/125
15.	Nagabala ghrita	Kshata khaya, Pittarakta	A.S.Chi5/69
16.	Amrita taila	Vata vyadhi chikitsa, Agnideepana	A.S.Chi.23/72
17.	Taila	Vatavyadhi chikitsa	A.S.Chi23/86
18.	Ghrita	Kshta Kshaya	A.Su.Chi 5/53
19.	Kashaya preparation	Pittaja Chardi	A.S.Chi.8/19
20.	Raja Yapana Basti	Aphordisiac / Vrishya	A.S.Ka.5/23
21.	Yapana Bati	Vrishya / Vitality	A.S.Ka5/34
22.	Medicated Oil	Vata Pitta diseases related to sukra, Rajas.	A.S.Ka.5/49
23.	Medicated Oil	Abhyanga	A.S.U.1/80
24.	Maha Kalyana Ghrita	Sannipaata vyadhis, Sthoulya	A.S.U.9/30
25.	Maha paishachaka ghrita	Unmada, Apasmara, Sannipata Jwara	A.S.U.9/32
26.	Medicated Oil	Paalitya Chikitsa	A.S.U.28/46
27.	Maha mayura Ghrita	Cures Different Indriya vyadhis, Sirorogas.	A.S.U.28/73
28.	Ksheera preparation	Vrishya , Visha	A.S.U.48/57
29.	Saradi Ghrita	Vrishya	A.S.U.50/26,30
30.	Beeja Churna with Ksheera	Vrishya	A.S.U.50/39,47.
31.	Svayam Gupta Churna Yoga	Vrishya	A.S.U.50/58,60
32.	Medicated Ghrita	Vrishya	A.S.U.50/62
33.	Kapikachu mulasidda Ksheera & Ghee	Vrishya	A.S.U.50/64
34.	Kapikachu sidha Poopalika with Ghrita	Vrishya	A.S.U.50/65, 68,69
35.	Padalepa Yoga	Vrishya	A.S.U.50/106, 108
36.	Medicated Ghee	Cough	A.H.Ci 3-38
37.	Amrita prasa ghrita	Cough,Hiccup	A.H.Ci 3/94-101; AFI (I)
38.	Svadamshtradi Ghrita	Vata pitta Hrid sula, Dysuria.	A.H.Ci.3/102-103
39.	Agastya Rasayana (Hareetaki Rasayana)	Rasayana,	A.H.Ci 3/127-132; AFI (I)
40.	Vashishta (Hareetaki) Rasayana	Rasayana,	A.H.Ci3/133
41.	Jeevantyadi Kalpa(Vaata pitta hara kalpa)	Asthapana vasthi (Retention enema)	A.H.Ka.4/60
42.	Medicated oil	Lepana for Karna pali Sandhana	A.H.Ut.40/17
43.	Beeja churna ,masha flour with Ksheera	Vrishya	A.H.Ut.40/31
44.	Kapikachu beeja churna , svadamshtra,ikshuras,satavari	Vrishya	A.H.Ut.40/34

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	with milk		
45.	Vidaryadi Ghrita	Vrishya	A.H.Ut.2/21,31,1/23
46.	Mahakalyan Ghrta	Kasa, Buddhidaurabalya, Apasmara, Vandhyatva, Jvara, Dourblya	A.H.Ut. 6/27-28, 32-33
47.	Kapikachu as diet	Vrishya	A.H.Su.6/22
48.	Balamuladi Nasya Chatushtayam	Vatavyadi	C.D.22-27
49.	Mashadi Kashaya (Maha Baladi kwath)	Vatavyadi	C.D.22.28
50.	Svaguptadi leha	Mutraghata	C.D.33/17,18
51.	Svayam guptadi churna	Vrishya	C.D.67/6
52.	Godhumadiya ghrita	Vrishya	C.D.67/26-36
53.	Gokshuraadi churna	Vrishya	C.D.66/26-34
54.	Brihad Aswagandhadhi ghrita	Vajikarana purpose	V.S.84/22-27
55.	Medicated Ghrita	Vajikarana purpose	V.S.84/65-66
56.	Gokshura Kapikachu beeja sidha ksheera	Vajikarana purpose	V.S.84/73
57.	Masha Ghrita	Vajikarana purpose	V.S.84/75-79
58.	Godhumaadi Ghrita	Vajikarana purpose	V.S.84/80-89
59.	Jeevanti Yamaka	Anuvasana, Nasya, Panaartha, urdhva jatrugata roga.	V.S.84/93-95
60.	Vanari gutika	Vajikarana	Y.R.Ut.11/27-31
61.	Kesar paka	Vajikarana	Y.R.Ut.11/49-56
62.	Rati modaka	Vajikarana	Y.R.Ut.11/57-60
63.	Kameswara modaka	Vajikarana	Y.R.Ut.11/68
64.	Kameswaar samdi paka modaka	Vajikarana	Y.R.Ut.11/77-83.
65.	Madana kamadeva rasa	Vajikarana	Y.R.Ut.11/117
66.	Rati vallabha pugapaka	Vajikarana	Y.R.Ut.11/61-67
67.	Satavaryadi churna	Vajikarana	Y.R.Ut.11/146
68.	Kapikachu paka	Vajikarana	Y.R.Ut.11/156-160
69.	Svayam gupta Ikshuraka yoga	Dhaatukshaya	B.R.70/14
70.	Gokshuradi yoga	Vajeekarana purpose	B.R 70/15
71.	Godhumadya ghritam	Vrishya purpose	B.R.70/18
72.	Brihadaswagandha ghritam	Vrishya purpose; Vandhyatva	B.R.70/19; AFI (II)
73.	Bricchatavari modaka	Vrishya purpose	B.R.70/21-24
74.	Rativallabha modaka	Vrishya purpose	B.R.70/25
75.	Mahakameswara modaka	Vrishya purpose	B.R.70/26
76.	Kamagni sandipana modaka	Vrishya purpose	B.R70/27
77.	Manmadhabhra rasa	Vrishya purpose; Rasayana; Vajikaran	B.R70/29; AFI (II)
78.	Mophara-Mopharava (Yavana krit yoga)	All diseases, consumed by kings Vrishya purpose.	B.R.70/30
79.	Masabaladi kvatha Churna	Pakshagata, Manyastambha, Karnanada, Arditavata	B.R.62-63 (Vatavyadhikara)
80.	Manikya rasa	Kustha; Vataroga; Jvara	B.R.300-308 (Kusthadhikara)
81.	Mrtasanjivanisura	Jvara; Dourblya; Karasya	B.R.694-703

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			(Javaradhikar)
82.	Brhatmasa Taila	Ardita; Apabahuk; Grdhrsi; Urdhva jatrugata roga	B.R.241-242 (Vatavyadhikara)
83.	Mashadi Ghritam	Vaajikaranartha	G.Ni.9-12
84.	Vajikaro payas Yoga	Vaajikaranartha	G.Ni.14
85.	Vajikaram Masha parnyadi churnam	Vaajikaranartha	G.Ni.16
86.	Vajikaram Swaguptadi churnam	Vaajikaranartha	G.Ni.19
87.	Vajikaram Swayamguptadi churnam	Vaajikaranartha	G.Ni.29
88.	Vajikaram Swayamguptadi Modakam	Vaajikaranartha	G.Ni.31-35,37.
89.	Vajikaram Sharmuladi Yoga	Vaajikaranartha	G.Ni.40-42
90.	Vajikari Godhumadi Pupalika	Vaajikaranartha	G.Ni.43-44
91.	Vajikaram Ikshurkadi Churnam	Vaajikaranartha	G.Ni.49.
92.	Visadi churna	Veerya vardhaka, Vajikara	H.Sa.47/9
93.	Guduchyadi Churna	Vajikara	H.Sa.47/13,14
94.	Kumaryasava	Jvara, Krmi, Daurbalya, Karsya, Ksaya, Mutrakrcchra	AFI (I)
95.	Vastyamayantaka Ghrita	Mutrakrcchra, Prameha, Ashmari	AFI (I)

Dosage of Kapikachu

Appropriate dosage of any drug is very important in the treatment of any disease. Physician should decide the dosage of any drug based on the patient condition age, strength, time, state of vitiated doshas and lastly disease.

Seed powder: 3-6 gms, **Pod hairs**-125mg, **Root decoction**: 50 – 100ml.[7,8]

Although, there are no varieties mentioned in respect of Kapikacchu, in Ayurveda. Fifteen species of *Mucuna* have been reported so far from India,out of which 3-4 varieties are in practice. These species are *Mucuna utilis* Wall. ex. Wight.[57] , *Mucuna cochinchinensis* (Lour.) A. Chev. (white variety) in the markets of Northern parts of India and *Mucuna cochichinensis* (Lour.) A.Chev (Black variety) used as Kapikachu in the Southern parts of the country, especially in Kerala.[58]

Recent studies have also shown that L-Dopa is the clinically active principle, which is used to cure Parkinson disease, to increase quality and quantity of sperm. Murthy *et.al.* (2015) study revealed that, a huge difference in the quantity of L-DOPA has been found among the 4 species *viz.Mucuna pruriens* (L.) DC., *Mucuna bracteata* DC., *Mucuna cochinchinensis* (Lour.) A. Chev. (black seed) and *Mucuna cochinchinensis* (Lour.) A. Chev. (white seeds). *M. cochinchinensis* black seed variety was found to be having maximum quantity of L-Dopa

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and also showed the antioxidant activity for reducing the possible and resultant damage of cellular constituents.[59] These findings validate the classical uses of Kapikachu, Vagbhata mentioned for its *rasayana* activity (rejuvenating property). Katzenschlager, *et.al*, 2004, clinically has reported, the seed powder formulation, containing *Mucuna* as a main ingredient was more effective than standard L-DOPA preparations, in the long term management of Parkinson disease.[24]

CONCLUSION

This article provides the knowledge of the drug passed on through ancient times till modern era. Different Samhitas and Nighantus highlighted the importance of synonyms, properties, classical uses and yogas (preparations) of Velvet bean. Various Yogas of Kapikacchu are being used to cure the diseases in all over the country. Not only the seed, but the whole plant *viz.* root, leaves, flower, pod hairs were also have certain medicinal properties. Hence, it offers a scope for further research to explore the therapeutic applications of all parts of drug, "Kapikachhu". Studies have also shown that natural source of L-DOPA, which is a precursor for dopamine, used in neurotransmitter diseases, is more potent than synthetic product. Moreover, it is necessary to exploit the total potential of the drug from different *Mucuna species*.

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REFERENCES

1. Nadkarni KM, Indian Materia Medica, Mumbai; Bombay Popular Prakashan, 1976; 818-820.
2. Anonymous, Indian Medicinal Plants, Varier's, P.S. Arya Vaidya shala, Kottakkal; Orient Longman Ltd, 1995; 4: 68-72.
3. Sharma P.C. Yelne MB, Dennis TJ, Database on Medicinal Plants used in Ayurveda, New Delhi; CCRAS, 2000; 1: 210-215.
4. Chopra RN, Nayar SL, Chopra IC, Glossary of Indian Medicinal Plants, New Delhi; CSIR, 2010; 171.
5. Gogate VM, Ayurvedic Pharmacology and Therapeutic Uses of Medicinal Plants (Dravyagunavidnyan), Mumbai; Bharatiya Vidhyabhavan (SPARC), 2000; 329-331.
6. Kirtikar KR, Basu BP, Indian Medicinal Plants, Allahabad; L.M. Basu, 1933; 1: 778-780.

WJPPS COPY PROOF

7. Nishteswar K, Hemadri K., Dravyaguna Vijnana, Detailed Study of the Drugs, Delhi; Choukhambha Sanskrit Pratishthan, 2013; 2: 43-46.
8. Sastry JLN, Dravya guna vijnana, VaranasiChowkhambha Orientalia, 2014; 2: 175-179.
9. Standley PC, Steyermark JA. Flora of Guatemala. Fieldian: Botany, 1946; 24(5).
10. Ding Y, Kinjo J, Yang C, Nohara, T. Triterpenes from Mucuna birdwoddiana. Phytochemistry, 1991; 30 (11): 3703-3707.
11. Daxenbichler ME, Van Etten CH, Hallman EA, Earle FR, Barclay AS. Seeds as sources of L-DOPA. J. Med. Chem. 1971; 14: 463.
12. Longhi JG., Perez E, Jose de Lima J, Candido LMB. In vitro evaluation of Mucuna pruriens (L.) DC. antioxidant activity. Brazilian Journal of Pharmaceutical Sciences, 2011; 47(3): 535-544.
13. Manyam BV. An alternative medicine treatment for Parkinson's disease: results of a multicenter clinical trial – HP-200 in Parkinson's disease study group. J. Alternat. Comp. Med., 1995; 1: 249-255.
14. Tan NH, Fung SY, Sim SM, Marinello E, Guerranti R, Aguiyi JC., The protective effect of Mucuna pruriens seeds against snake venom poisoning, J Ethnopharmacol, 2009; (123): 356–358.
15. Fung SY, Tan NH. (2012), Effect of Mucuna pruriens seed extract pretreatment on the responses of spontaneously beating rat Atria and Aortic Ring to Naja sputatrix (Javan Spitting Cobra) Venom. Journal Evidence-Based Complementary and Alternative Medicine, 2012; (2012), (http://dx.doi.org/10.1155/2012/486390).
16. Vadivel V, Janardhan K, Nutritional and antinutritional composition of velvet bean: an under-utilized food legume in South India. International Journal of Food Science Nutrition, 2000; 51: 279-287.
17. Bhaskar A, Vidhya VG, Ramya M, Hypoglycemic effect of Mucuna pruriens seed extract on normal and streptozotocin-diabetic rats, Fitoterapia, 2008; 79(7-8): 539-543.
18. Shukla KK, Mahdi AA, Ahmad MK, Jaiswar SP, Shankwar SN, Tiwari SC., Mucuna pruriens Reduces Stress and Improves the Quality of Semen in Infertile Men. Evidence Based Complementernative and Alternative Medicine, 2007; 7(1): 137-144.
19. Jadhav SR. Physiological study of Shukravaha Srotas and clinical study of kapikacchu Churna in Klaibya with special Ref. to oligozoospermia" (Thesis), PG Dept. Of Sharir kriya, Jaipur: National Institute of Ayurveda, 2013; 141-145.
20. Jadhav SR, Dadhich OP, Kothari P., KAPIKACCHU (Mucuna pruriens) - A Ayurvedic drug review, World J Pharm Sci., 2015; 3(10): 1999-2003.

WJPPS COPY PROOF

21. Shukla KK, Mahdi AA, Ahmad MK, Shankhwar SN, Rajender S, Jaiswar SP., Mucuna pruriens improves male fertility by its action on the hypothalamus pituitary gonadal axis. Fertil Steril, 2009; 92(6): 1934-40.
22. Rajeshwar Y, Gupta M, Mazumder UK., Antitumor Activity and in vivo Antioxidant Status of Mucuna pruriens (Fabaceae) Seeds against Ehrlich Ascites Carcinoma in Swiss Albino Mice. Iranian Journal of Pharmacology & Therapeutics, 2005; 4(1): 46-53.
23. Hussain, G, Manyam BV. Mucuna Pruriens proves more effective than L-DOPA. In Parkinson's disease animal model, Phytotherapy Research, 1997; 11(6): 419-423.
24. Katzenschlager R, Evans A, Manson A, Mucuna pruriens in Parkinson's disease: a double blind clinical and pharmacological study. Journal of Neurology, Neurosurgery and Psychiatry 2004; 75: 1672–1677.
25. Manyam BV, Dhanasekaran M, Theodore AH, Neuroprotective, Effects of the Antiparkinson Drug Mucuna pruriens, Phytotherapy Research, 2004; 18: 706–712.
26. Patil D, Pandey DK, Mahesh R, Kurdekar V, Jhadav HR. Anti-Depressant-Like Activity of Mucuna Pruriens; A Traditional Indian Herb in Rodent Models of Depression, Pharmacology online, 2010; (1): 537-551.
27. Eze JI, Ndukwe S. Effect of methanolic extract of Mucuna pruriens seed on the immune response of mice. Comp Clin Pathol, 2012; 21: 1343–1347.
28. Natarajan K, Narayanan N, Ravichandran N. Review on "mucuna" - the wonder plant, Int. J. Pharm. Sci. Rev. Res, 2012; 17(1): 86-93.
29. Sharma PV. "Charaka Samhita", Varanasi; Chaukhambha Oriantalia: 2000; 1.
30. Sharma PV. "Susruta Samhita", Varanasi; Chaukhambha Vishvabharati: 1999; I.
31. Srikantha Murthy. Vagbhata's "Astanga Sangraha", Varanasi; Chaukhambha Orientalia: 2001; 1.
32. Srikantha Murthy, Vagbhata's "Astanga Hrdayam", Varanasi; Krishnadas Academy: 1999; 1.
33. e- nighantu. National Institute of Indian Medical Heritage, Hyderabad, http ://niihm.nic.in (Accessed on 5th December, 2015).
34. Pandit Hariprasad Tripathy. "Dhanvantari Nighantu", Varanasi; Chowkhambha krishnadas Academy: 2008; 42
35. Sharma PV. "Kaiyadev Nighantu", translated by Guru prasad Sharma, Varanasi ; Chaukhambha Orientalia: 1979, Oushadhi varga 111-154.
36. Srikantha Murthy. "Bhavaprakasa" of Bhavamisra, Varanasi; Krishnadas Academy: 1998; 1, Guduchydi varga-57, 6/4-129/131.

WJPPS COPY PROOF

37. Indradev Tripathi. "Raja Nighantu" of Pandit Narhari, Varanasi; Chowkhambha Krishnadas Academy: 2010; 38.
38. Pandey Gyanendra. "Sodhala Nighantuh", Varanasi; Chowkhambha Krishnadas Academy: 2009; 40.
39. Sastry JLN. "Madanpal Nighantu" by Nrupamadanpal, Bombay; Laxmivyankateshwar Steam Press, kalian: 1954, Abhayadivarga Shlok.70-71; 225-226.
40. Sharma PV. "Priyanighantuh", Varanasi; Chokhambha Sanskrit Pratishthan: 2004; 66.
41. Vaidya B. "Nighantu Adarsha", Varanasi; Chaukhambha Bharati Academy: 1998, Palashadi Varga, 2; 453-456.
42. Anonymous, The Ayurvedic Pharmacopoeia of India, 1st edn., New Delhi; Ministry of Health & Family Welfare. Department of Indian Systems of Medicine & Homeopathy: 2001; 1(3): 23-24.
43. Sharma PV. "Charaka Samhita", Varanasi; Chaukhambha Oriantalia: 2000; 2.
44. Sharma PV. "Susruta Samhita", Varanasi; Chaukhambha Vishvabharati: 2000; II.
45. Srikantha Murthy. Vagbhata's "Astanga Hrdayam", Varanasi; Krishnadas Academy: 2000; 3.
46. Sharma PV., "Cakradatta", Varanasi; Chaukhambha Oriental, 1994, Vrushyadhikara, 66/6, 9; 26-34.
47. Pandit Hariprasad Tripathy. "Vangasen Samhita", Varanasi; Chowkhambha Sanskrit Series office : 2009.
48. Madham Shetty Suresh Babu, "Yoga Ratnakar", Vol-1 & 2, Varanasi; Chowkhambha Sanskrit Series.
49. Pandey Gyanendra . "Bhaisajya Ratnavali", Varanasi, Chowkhambha Sanskrit Series office: 2005.
50. Sri Indradeva Tripathi, edn Sri Gangasahaya Pandeya "Gadanigraha" of Sri Vaidya Sodhala, Part-2, Kayachikitsa Khanda, Varanasi; Chaukhambha Sanskrut Sasthan: 2011.
51. Pandit Hariprasad Tripathy. "Harita Samhita", Varanasi; Chowkhambha Krishnadas Academy: 2009.
52. Chunekar KC. Bhavprakasa Nighantu, Indian Materia Medica of Sri. Bhavamisra, Varanasi ; Chaukhambha Bharati Academy: 2013; 342-343.
53. Narayan Das. "Rajvallabha Nighantu" Dravya Chandrika Tikasahit, Gangavishnu Krishnadas Mumbai; Laxmivyankateshwar' Steampress: 1952.
54. Saligram Vaishya,. "Saligramnighantu Bhushanam (Brihatnighantu Ratnakarantargat 7-8 part), Mumbai; Khemraj ShriKrishnadass, 258-259.

WJPPS COPY PROOF

55. Anonymous. The Ayurvedic Formulary of India, 2nd revised edn., New Delhi; Ministry of Health & Family Welfare. Department of Indian Systems of Medicine & Homeopathy: 2003 Part I: 10-11; 18; 33; 79; 94; 97-98; 146-147.
56. Anonymous. The Ayurvedic Formulary of India, 1st edn., New Delhi; Ministry of Health & Family Welfare. Department of Indian Systems of Medicine & Homeopathy, 2000; II: 79; 100-101; 275; 279.
57. Garg, Sunita. Substitute and Adulterant Plant, New Delhi; Periodical Expert Book Agency: 1992; 82.
58. Lucas DS. Dravyagun Vijnana study of Dravya-Materia Medica, Varanasi; Choukhambha Bharati Academy: 2013; 2: 123-126.
59. Murthy S N, Manasi M. Malgaonkar, A. R. Shirolkar, S. D.Pawar, S. Sangvikar, Y.R. Kulkarni. A comparative assessment of pharmacologically active principles and antioxidant activity of commonly occurring Mucuna sps. in India. International Journal of Ayurveda and Pharma Research, 2015; 3(11): 8-13.

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Research Article

A COMPARATIVE ASSESSMENT OF PHARMACOLOGICALLY ACTIVE PRINCIPLES AND ANTIOXIDANT ACTIVITY OF COMMONLY OCCURRING *MUCUNA SPS.* IN INDIA

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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 (Leguminoceae) 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, anthelminitc, alexipharmic, hypotensive, spasmodic, hypocholestrolemic, 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, powered 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 stembark 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 [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50ºC 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 ($FeCl_3$) (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 *M 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.	*Mucuna cochinchinensis* black seed	190.7156 ± 4.2161
2.	*Mucuna cochinchinensis* white seed	96.2282 ± 3.9161
3.	*Mucuna pruriens*	77.6826 ± 3.1232
4.	*Mucuna bracteata*	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	*M. cochinchinensis* black seed extract	*M. cochinchinensis* white seed extract	*M. pruriens* extract	*M. bracteata* 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 spermatogensis 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 facililation 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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REFERENCES

1. Eilittä M, Bressani R, Carew LB, Carsky RJ, Flores M, Gilbert R, Huyck L, St-Laurent L, Szabo NJ. Mucuna as a food and feed crop: an overview. In: Mucuna as a Food and Feed: Current Uses and the

Way Forward. Ed. By Flores M, Eilittä M, Myhrman R, Carew L, Carsky R, Workshop held April 26-29, 2000 in Tegucigalpa, Honduras. CIDICCO Honduras. 2002; pp. 18-46.

2. Janardhanan K, Lakshmanan KK. Studies on the pulse, Mucuna utilis. Chemical composition and anti-nutritional factors. J. Food Sci. Tech. 1985; 22: 369-37.
3. Mohan VR, Janardhanan K. Studies on the Indian tribal pulses. In proceedings of the national seminar on biodiversity: strategies for conservation and future challenges. Department of Botany. Bharathiar University. Ed. by Udaiyan K, Janardhanan K, Manian S, Reddy VRKP 1993; pp. 116-125.
4. Manyam BV. An alternative medicine treatment for Parkinson's disease: results of a multicenter clinical trial – HP-200 in Parkinson's disease study group. J. Alternat. Comp. Med. 1995; 1, 249-255.
5. Standley PC, Steyermark JA. Flora of Guatemala. Fieldian: Botany, 1946; Vol 24 Part V.
6. Ding Y, Kinjo J, Yang C, Nohara T. Triterpenes from Mucuna birdwoddiana. Phytochemistry, 1991; 30(11), 3703-3707.
7. Sastry CST, Kavathekar YY. Plants for reclamation of wastelands. Publications and Information Directorate, New Delhi. 1990; pp 317-318.
8. Singh U, Wadhwani AM, Johri BM. Dictionary of economic plants in India. Indian Council of Agricultural Research, New Delhi. 1996; pp. 45-46.
9. Anonymous. The Wealth of India Raw Materials, NISCAIR Publishing New Delhi, L-M; VI, 2005; 439-444.
10. Desai SM., Sharon M, Sharan M. Effect of Culture Conditions on L-Dopa Accumulation in Callus Culture of Mucuna pruriens. J. Chem. Pharm. Res, 2010; 2(4), 134-146.
11. Lowry OH, Rosebrough NR, Lewis Farr A, Randall RJ. Protein measurement with the Folin Phenol Reagent, J. Biol. Chem, 1951; 193, 265-275.
12. Khatiwora E, Adsul VB, Kulkarni MK, Deshpande NR, Kashalkar RV. Spectroscopic determination of total phenol and flavonoid contents of Ipomoea carnea. International Journal of ChemTech Research, 2010; 2 (3), 1698-1701.
13. Slinkard K, Singleton V.L. Total phenol analyses; automation and comparison with manual methods. Am. J. Enol. Vitic. 1977; 28, 49-55.
14. Blois, M.S. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181, 1199-1200.
15. Oyaizu, M. Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition. 1986; 44, 307-315.
16. Daxenbichler ME, Van Etten CH, Hallman EA, Earle FR, Barclay AS. Seeds as sources of L-DOPA. J. Med. Chem. 1971; 14, 463.
17. British Pharmacopoeia. (1973), London.
18. Longhi JG, Perez E, Jose de Lima J, Candido LMB. In vitro evaluation of Mucuna pruriens (L.) DC. antioxidant activity. Brazilian Journal of Pharmaceutical Sciences, 2011; 47(3), 535-544.
19. Singh AP, Sarkar S, Tripathi M, Singh R. Mucuna pruriens and Its Major Constituent L-DOPA Recover Spermatogenic Loss by Combating ROS, Loss of Mitochondrial Membrane Potential and Apoptosis. PLoS One. 2013; 8(1), e54655.
20. Malmnas CO. The significance of dopamine, versus other catecholamines, for L-dopa induced facilitation of sexual behavior in the castrated male rat. Pharmacol. Biochem. Behav., 1976; 4(5), 521-526.
21. Gulcin I. (). Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa. Amino Acids, 2007; 32, 431–438.
22. Gow-Chin Y, Chiu-Luan H. Antioxidant effects of dopamine and related compounds. Biosci Biotech Biochem, 1997; 61 (10), 1646–1649.
23. Kazuki K, Hiroyuki S. High content of dopamine, a strong antioxidant, in Cavendish banana. J Agric Food Chem, 2000; 48, 844–848.

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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.

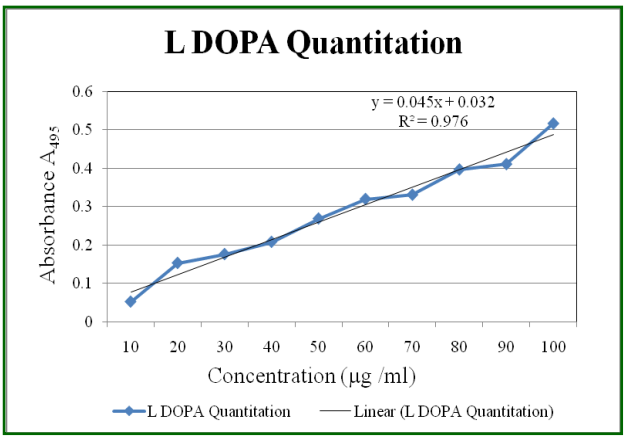


Figure a: The standard plot of L-dopamine

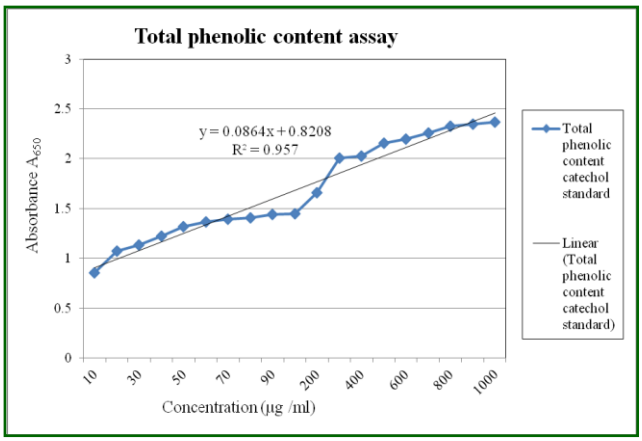


Figure b: The standard plot of total phenolic content assay

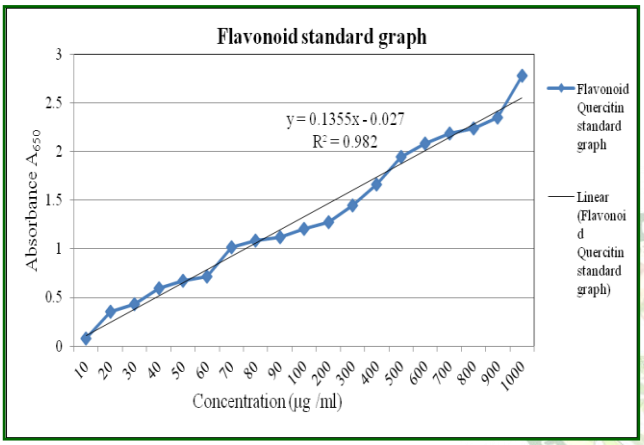


Figure c: The standard plot of flavonoid assay

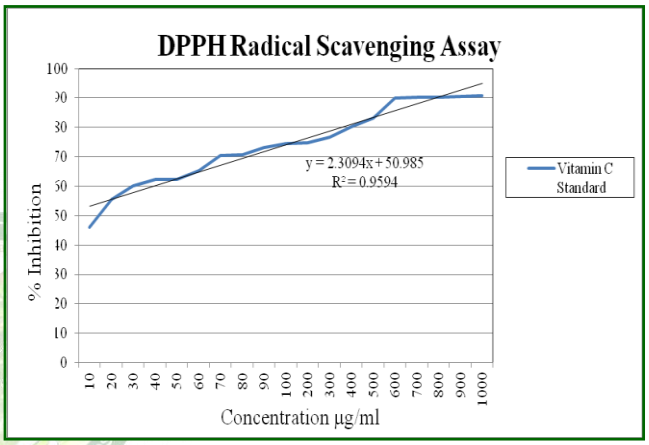


Figure d: The standard plot of DPPH radical scavenging assay

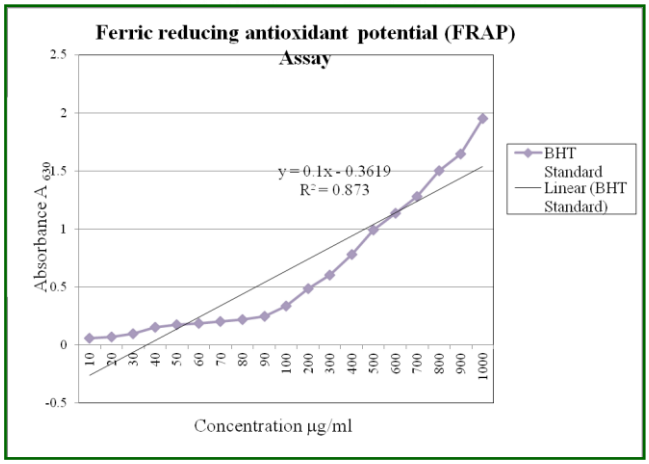


Figure e: The standard plot of FRAP assay

COMPARATIVE ASSESSMENT OF SEMEN PROFILE AND BIOCHEMICAL PARAMETERS AFTER TREATMENT WITH *MUCUNA* SPS. IN SPRAGUE DAWLEY RATS

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ABSTRACT

The different varieties of Kapikachu viz., *Mucuna cochinchinensis* (Lour.) A Chev. (Black seed variety), *M. cochichinensis* (Lour.) A Chev. (White seed variety) and *M. pruriens* (L.) DC. are used in the treatment of infertility in India by Ayurvedic physicians. The study was carried out for comparative assessment of their effect on semen profile and biochemical parameters in Sprague Dawley strain rats. The animals were treated with different dose of aqueous extract of *M. cochinchinensis* (Black seed variety), *M. cochichinensis* (White seed variety) and *M. pruriens* for the period of 30 days. The body weight, sperm count, sperm motility, morphology of sperm, serum levels of testosterone, estradiol, Luteinizing hormone (LH), and Total antioxidant capacity were measured. The testes were isolated, weighed and sectioned to study the histological changes. The results of the study showed the increase in sperm count, serum motility, levels of serum testosterone, LH and total antioxidant capacity in animals treated with aqueous extract of *M. cochinchinensis* (Black seed variety), followed by *M. pruriens and M. cochichinensis* (White seed variety). These observations were supported by histological change in testes. From the results obtained, it can be concluded that the *M. cochinchinensis* (Black seed variety) is more potent than other varieties of Kapikachu in rats.

Keywords: Spermatogenesis, *Mucuna cochinchinensis* (Lour.) A. Chev., *Mucuna pruriens* (L.) DC., L-dopa

INTRODUCTION

Ayurveda a traditional health care system believes in the philosophy of not only treating the diseases but also to help in maintaining the healthy state of every individual with the help of naturally available resources such as commonly available herbs, spices, minerals and animal products. Vrishya chikitsa is one of the eight branches of Ayurveda which is devoted to the treatment of infertility, impotence and other diseases related to the reproductive system in males.

Vrishya (Aphrodisiac) therapy helps in nourishing the body tissues of an individual with a target of improving the quality of germinative tissues. A drug or therapy which bestows off spring, immediate feeling of joy, the capacity to copulate with women with great vigour like a horse and becoming dearer to women thereby is known as Vajeekarana (Virilification)[1](A.S.Utta. Chapt 50/1-3). Kapikachu being known for its Vajeekarana activity, is selected in this study for assessment of its effect on spermatogenesis. Susruta used this for the first time for vajeekarana purpose. Later on, the authors of all the Nighantus also mentioned this drug for vrishya chikitsa.

Recent studies also suggested that oral administration of *Mucuna pruriens* (L.) DC seed powder helps in reducing psychological stress and seminal plasma lipid peroxide levels along with improved sperm count and motility[2]. In another study has shown significant improvement in terms of sperm count, volume, and motility of sperm. It has shown mild improvement in non progressive sperm (NP) and no improvement in slow linear progress of (SLP) sperm[3,4]. In some other study *M. pruriens* and its major constituent L-Dopa has recovered the spermatogenic loss by combating ROS, loss of mitochondrial membrane potential and apoptosis[5].

The present study was therefore aimed for comparative assessment of semen profile and biochemical parameters in Sprague Dawley rats using method described by Khaki *et al*.[6] after treatment with different varieties of Kapikachu *viz*., *Mucuna cochinchinensis* (Lour.) A. Chev (Black seed variety), *Mucuna cochichinensis* (Lour.) A. Chev (White seed variety) and *Mucuna pruriens* (L.) DC. in Sprague Dawley strain rats.

MATERIALS AND METHODS

Collection of plant material

Healthy, fresh and disease free plant material - seeds of *Mucuna pruriens* (L.) DC. and *Mucuna*

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cochinchinensis (Lour.) Chev. (White seed) were collected from NRIBAS, garden and local market of Pune (Maharashtra) respectively. *Mucuna cochinchinensis* (Lour.) Chev. (Black seed) collected from local market of Kerala.

Authentication of plant material

Crude drug were identified and authenticated by Agharkar Research Institute, Pune and deposited with voucher number, S-162 (*Mucuna pruriens* (L.) DC.), S-159 (*Mucuna cochinchinensis* (Lour.) A. Chev. (White seed)) and S-159 (*Mucuna cochinchinensis* (Lour.) A. Chev. (Black seed)).

Elemental study

Elemental studies of the samples collected from the market and fields were carried out in detail as per the standard methods. Analysis was outsourced to Maharashtra Rajya Draksha Bagaitdar Sangh (MRDBS), Manjari Farm, Pune.

Elemental study parameters

Sr. No.	Analysis Parameter	Method for Analysis
1	Total Nitrogen	Wet Digestion Followed by Kjeldahl distillation Method
2	Nitrate Nitrogen	Phenol Disulphonic Acid Method
3	Digestion	Diacid digestion (HNO_3 : $HClO_4$) – 3:1
4	Phosphorous	Spectrophotometric Vanado Phospho Molybdate Method
5	Potassium	Flame Photometric Method
6	Calcium	Atomic Absorption Spectrophotometric Method
7	Magnesium	Atomic Absorption Spectrophotometric Method
7	Sulphur	Barium Chloride, Turbidimetric Method
9	Iron	Atomic Absorption Spectrophotometric Method
10	Manganese	Atomic Absorption Spectrophotometric Method
11	Zinc	Atomic Absorption Spectrophotometric Method
12	Copper	Atomic Absorption Spectrophotometric Method
13	Sodium	Flame Photometric Method

Preparation of extracts:

The extract was prepared as mentioned in Murthy *et al*.[7]. The crushed seeds powders of *Mucuna* sps were immersed in water and the flasks were kept at 40°C in a 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 concentrate further. The dried, powered forms of these extracts were preserved at dark and cold place to maintain the medicinal value till further use.

Experimental animals:

Sixty six Sprague Dawley strain male rats were procured and acclimatized for one week in experimental room after veterinary examination. They were housed in clean poly propylene cages with stainless steel grill top, facilities for food and water bottle, with the bedding of clean paddy husk. The environmental conditions such as air conditioned experimental rooms with 10-15 air changes per hour, temperature between 21±2ºC, relative humidity 55±5% and illumination cycle set to 12 hours fluorescent light and 12 hours dark was maintained for animal house facility. Diet of the animals was "Nutrimix" brand pelleted standard rat feed Manufactured by Nutrivet Life Sciences, Pune was provided *ad libitum*. Potable water passed through reverse osmosis filtration system and exposed to U.V. ray was provided *ad libitum* in glass bottles with stainless steel sipper tubes. Sixty six male rats weighting between 250-270 gm were used.

Ethical clearance:

The study was carried out at Toxindia Contractual Research Organization, Pune, after obtaining approval of Institutional Animal Ethics Committee (IAEC) meeting held on 26th September 2013, with approval code number TI003 as per guidelines of the CPCSEA.

Experimental design:

Male rats were divided into 11 groups (Group I-XI) comprising of six animals in each group. Group I (Control) received distilled water and normal rat chow. Group II (Standard) received L-Dopa at 20 mg/kg orally. While Group III, IV, and V received 100, 200 and 400 mg/kg of *M. cochinchinensis* (Black seed variety) aqueous extract orally, respectively. Similarly, Group VII, VIII, and VIII received 100, 200, and 400 mg/kg of *M. cochichinensis* (White seed variety) aqueous extract orally, respectively. Group IX, X, and XI received 100, 200, and 400 mg/kg of *M. pruriens* aqueous extract orally. All the animals were

Table 1: Elemental analysis of *Mucuna sps.*

Sr. No.	Parameters	M. pruriens (L.) DC.	M. cochinchinensis (Lour.) A. Chev. (White Seed)	M. cochinchinensis (Lour.) A. Chev. (Black Seed)
1	Nitrogen	5.22%	4.25%	4.61%
2	Phosphorus	0.35%	0.32%	0.32%
3	Potassium	1.15%	1.20%	1.10%
4	Calcium	0.024%	0.016%	0.020%
5	Magnesium	0.19%	0.16%	0.18%
6	Sulphur	0.29%	0.26%	0.21%
7	Iron	19ppm	18 ppm	15ppm
8	Manganese	29ppm	5 ppm	16ppm
9	Zinc	40ppm	34 ppm	32ppm
10	Copper	18ppm	19 ppm	15ppm
11	Sodium	0.25%	0.20%	0.20%

Table – 2: Group mean body weight (gm)

Group	Dose mg/kg	Sample		0	7	14	21	28	30
I	0	Control	Mean	260.67	274.33	287.50	301.67	315.33	320.17
			±SD	8.55	9.14	9.48	9.61	9.95	10.89
II	20	L-Dopa	Mean	261.00	287.67	302.33	317.17	332.00	340.33
			±SD	8.65	6.95	7.37	7.78	7.07	6.06
III	100	Mucuna cochinchinensis (Black seed)	Mean	258.33	269.83	282.00	294.00	305.67	309.83
			±SD	9.07	7.96	8.07	7.87	8.52	8.40
IV	200		Mean	260.33	272.50	285.50	298.00	310.17	314.67
			±SD	3.88	4.09	4.09	4.15	4.17	3.93
V	400		Mean	258.67	279.17	289.67	309.33	321.17	337.17
			±SD	7.97	5.49	3.56	12.21	6.68	7.86
VI	100	Mucuna cochinchinensis (White seed)	Mean	261.00	273.33	286.50	299.33	312.33	316.67
			±SD	5.48	5.39	5.68	6.12	6.22	6.65
VII	200		Mean	259.33	271.67	284.00	296.50	308.33	312.00
			±SD	6.65	6.68	6.39	5.92	6.02	5.37
VIII	400		Mean	260.00	271.50	283.83	296.50	307.67	311.17
			±SD	8.20	8.29	8.08	7.97	7.97	7.52
IX	100	Mucuna pruriens	Mean	261.33	273.83	286.67	299.83	312.50	318.33
			±SD	5.16	5.04	5.09	5.38	5.89	6.65
X	200		Mean	260.00	273.00	286.33	299.67	312.83	320.17
			±SD	5.80	5.80	5.82	5.82	6.01	6.82
XI	400		Mean	261.33	283.83	294.00	315.17	324.17	331.17
			±SD	5.16	4.54	4.00	5.71	3.19	9.15

treated orally with the drug for 30 days. The weekly body weight of all the animals was measured upto 30 days of the experimental period.

Collection and processing of blood samples:

Blood samples were collected from all the animals by orbital bleeding from ether anesthetized animals after 30 days of the treatment. Approximately 1-1.5 ml of blood was collected from all rats in each group including the control group. The samples were carefully introduced into containers free from anticoagulant and properly labeled. The blood samples were allowed to clot, retract and then centrifuged for 5 minutes at a speed of 5000 revolutions per minute.

Biochemical Analysis:

The collected serum was refrigerated at -20°C to carry out testosterone, estradiol, Luteinizing hormone (LH), and Total Antioxidant Capacity (TAC) analysis using kit based assays (Biovision, California, US).

Table -3 Effect of treatment on weight of testes, epididymis, sperm count, sperm motility, sperm morphology in rats.

Parameters	Group & Dose (mg/kg)	Control	L-Dopa	M. cochinchinensis (Black seed variety)			M. cochinchinensis (White seed variety)			M. pruriens		
		G1 0	G2 20	G3 100	G4 200	G5 400	G6 100	G7 200	G8 400	G9 100	G10 200	G11 400
Weight of Testes (Absolute)	Mean	2.890	3.298	2.901	3.101	3.294	2.827	2.883	2.936	2.899	2.915	3.220
	±SD	0.27	0.20	0.18	0.17	0.09	0.34	0.14	0.16	0.23	0.18	0.23
Weight of Testes (Relative)	Mean	0.902	0.975	0.912	0.969	0.965	0.894	0.925	0.944	0.936	0.926	0.931
	±SD	0.07	0.04	0.06	0.06	0.04	0.12	0.06	0.05	0.08	0.06	0.07
Weight of Epididymis (Absolute)	Mean	0.70	0.88	0.74	0.77	0.87	0.69	0.70	0.70	0.70	0.70	0.84
	±SD	0.06	0.04	0.03	0.02	0.05	0.05	0.05	0.05	0.05	0.06	0.10
Weight of Epididymis (Relative)	Mean	0.220	0.250	0.233	0.240	0.245	0.217	0.226	0.226	0.225	0.224	0.228
	±SD	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.01	0.02	0.01
Sperm count (10^6/ml)	Mean	20.57	28.83	20.73	21.77	26.48	19.57	20.20	20.27	19.72	20.37	26.00
	±SD	1.45	1.96	2.97	2.11	2.99	2.07	2.01	2.13	1.85	2.64	2.15
Sperm motility (%)	Mean	81.92	90.58	78.92	81.58	89.67	69.92	73.33	80.58	80.67	77.75	88.50
	±SD	3.47	4.16	5.94	6.09	2.09	5.38	6.62	2.92	3.19	5.39	2.74
Sperm morphology (%)	Mean	77.57	85.50	75.60	79.80	84.90	69.83	72.20	75.33	70.13	73.57	84.00
	±SD	2.32	2.51	5.11	3.50	2.20	3.79	5.53	5.87	5.24	4.35	0.89

Table 4: Biochemical rat serum levels of Estradiol, Teststerone, Luteinizing Hormone and Total Antioxidant Capacity in rats treated with aqueous extract of *Mucuna* sps (400 mg/kg body weight)

Sl. No.	Name of chemical test/assay	Control	L-Dopa	M. cochinchinensis (Black)	M. cochinchinensis (White)	M. pruriens
1	Estradiol EIA Assay	9.548±0.68	8.654±0.64	7.49±0.57	7.996±0.51	7.669±0.56
2	Testosterone EIA assay	225.42±10.35	380.55±11.55	377.14 ±19.55	338.15±10.92	350.84±16.55
3	Luteinizing hormone EIA assay	1.04±0.139	1.575±0.138	1.367±0.158	1.288±0.142	1.453±0.145
4	Total Antioxidant Capacity Assay	13.31±1.92	13.87±1.55	15.232±1.81	14.991±1.67	21.066±1.86

Histopatholgical study:

After completion of experimental duration, animals were sacrificed and testes were isolated and absolute and relative weight of testes and epididymis was measured. The sperm count, motility and morphology of sperm in all the animals were noted. The isolated testes were kept in specimen bottle containing Bouin fluid solution and processed for paraffin sectioning. Sections of 5µ thickness were prepared and stained with haematoxylin and eosin for microscopic (histology) study of testes.

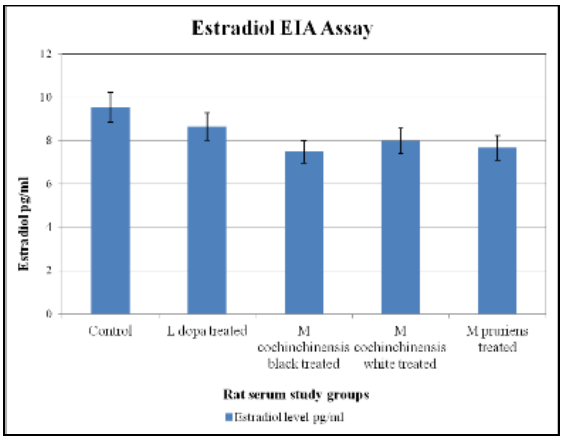
Statistical analysis:

The results were expressed as mean ± SEM. The significance of differences was analyzed using Student's t-test, $p<0.05$ was considered as significant.

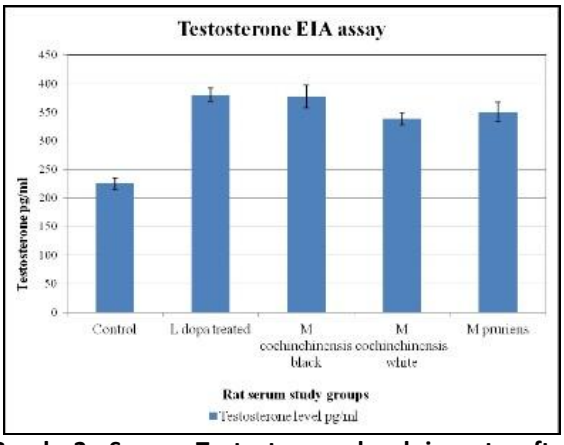
RESULTS AND DISCUSSION

Elemental Analysis:

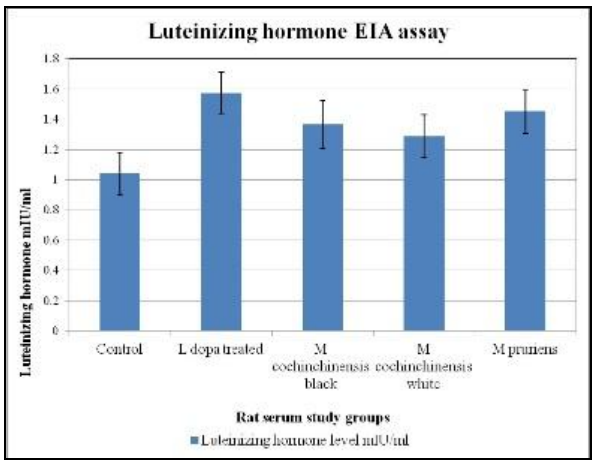
The elemental analysis showed that significant levels of zinc, manganese and calcium in seeds of *M. pruriens* followed by *M. cochinchinensis* black



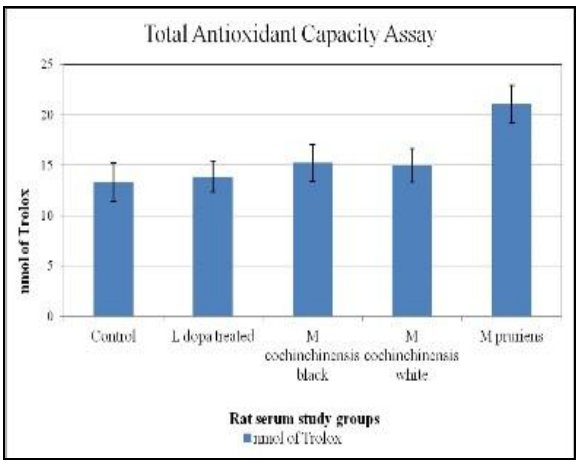
Graph 1: Serum Estradiol level in rat after treatment with aqueous extract of *Mucuna sps.* (400 mg/kg body weight)



Graph 2: Serum Testosterone level in rats after treatment with aqueous extract of *Mucuna sps.* (400 mg/kg body weight)



Graph 3: Serum LH level in rat after treatment with aqueous extract of *Mucuna sps* (400 mg/kg body weight)



Graph 4: Serum TAC level in rat after treatment with aqueous extract of *Mucuna sps* (400 mg/kg body weight)

and white seed variety. The elemental analysis of seeds of *Mucuna* sps is depicted in Table 1.

Animal study:

There was no incidence of treatment related mortality among the animals exposed to the standard drug and test materials (*M. cochinchinensis* (Black seed variety), *M. cochichinensis* (White seed variety) and *M. pruriens*) at 100, 200, 400 mg/kg body weight. Treatment of rats with test materials at all doses did not induce any noticeable treatment related abnormal clinical signs.

Body Weights:

M. cochichinensis (Black seed variety) and *M. pruriens* at 400 mg/kg body weight dose and standard drug induce a significant increase in the group mean body weights of the animals. Other doses at 100 and 200 mg/kg body weight of test drugs did not induce any observable change in body weight animals (Table 2).

Food Consumption:

The values of average daily food consumption in all groups were comparable with control group of animals.

Necropsy:

All test materials (*M. cochinchinensis* (Black seed variety), *M. cochichinensis* (White seed variety) and *M. pruriens*) at doses of 100, 200 and 400 mg/kg body weight and standard drug at 20 mg/kg dose did not induce any significant treatment related gross histological changes in any of the organs or tissues of treated rats.

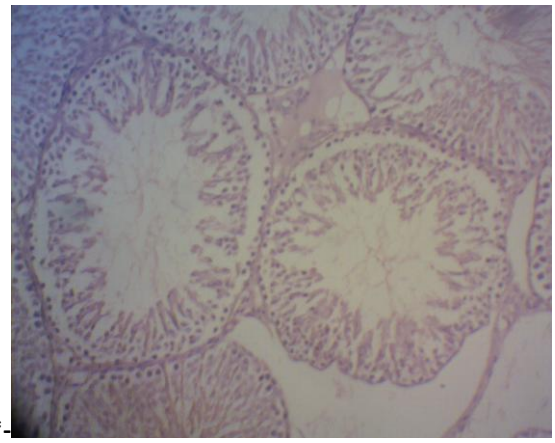


Fig 1. TS of rat testes in Control Group.

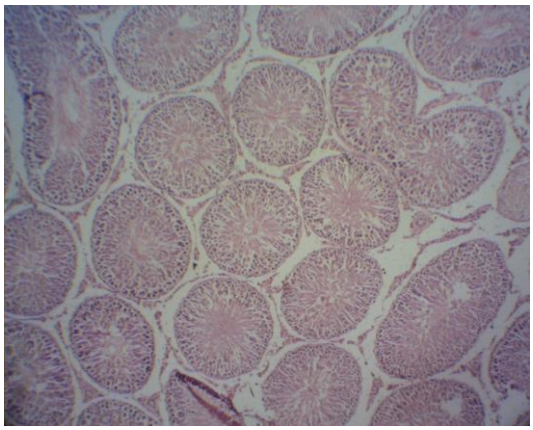


Fig 2. TS of rat testes treated with aqueous extract of *Mucuna cochinchinensis* (Black seed) at 400 mg/kg body weight, orally.

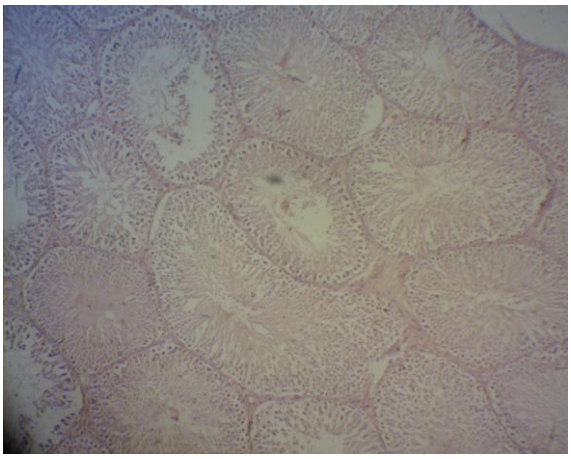


Fig 3. TS of rat testes treated with aqueous extract of Mucuna pruriens at 400 mg/kg body weight, orally.

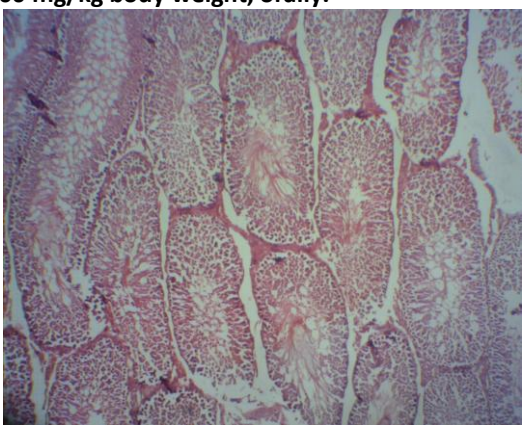


Fig 4. TS of rat testes treated with aqueous extract of Mucuna cochinchinensis (White seed) at 400 mg/kg body weight, orally.

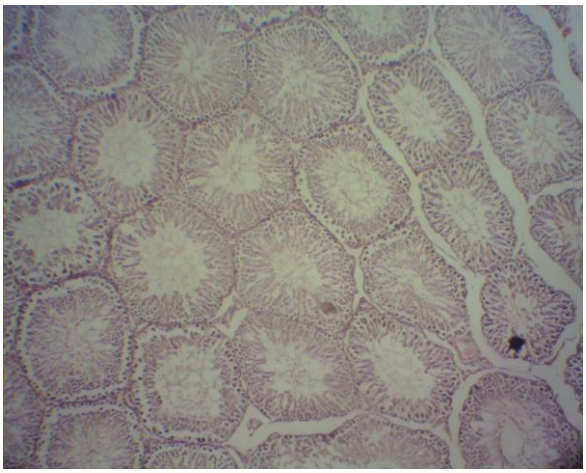


Fig 4. TS of rat testes treated with L-Dopa at 20 mg/kg body weight, orally.

Weights of Organs:

Increase in absolute and relative weights of testes and epididymis in group II, VIII and XI animals was found to be statistically significant ($p<0.05$) when compared with control group of animals (Table 3).

Sperm count, motility and morphology of sperm:

Treatment with the standard drug at 20 mg/kg and *M. cochichinensis* (Black seed variety) and *M. pruriens* at 400 mg/kg body weight for 30 days induces a significant increase in sperm count,

motility and morphology of sperms ($p<0.05$) (Table 3).

Biochemical Analysis:

The rat serum levels of testosterone, luteinizing hormone and total antioxidant capacity showed significant increase after treatment with the aqueous extracts of *M. cochinchinensis* (Black seed variety), *M. cochichinensis* (White seed variety) and *M. pruriens* at 400 mg/kg body weight for 30 days as compared to the normal control animals. The increase in serum levels of these biochemical parameters was comparable with those of L-Dopa treated animals. The level of rat serum estradiol in the treatment group was declined as compared to the normal control animals (Table 4 and Graph 1-4).

Histological section:

The histological cross sections of testes in the control group showed normal histological texture (Fig 1). Cross section of testes in the aqueous extract treated groups with 400 mg/kg body weight of *M. cochinchinensis* (black seed variety) revealed more number of seminiferous tubules having maximum diameter, large number of different cells (sertoli, leydigs cells) at various stages of spermatogenesis and spermatozoa in seminiferous tubules (Fig 2). This profile was followed by sections of testes treated with *M. pruriens* and *M. cochinchinensis* white seed variety (Fig 3-4).

DISCUSSION

In spermatogenesis and recovery of endocrine axis, the role of L-3, 4-dihydroxy phenylalanine (L-Dopa) is well known from the earlier studies. L-dopa, a non-protein amino acid, stimulates the hypothalamus and forebrain which leads to the production of gonadotropin releasing hormone. Gonadotropin releasing hormone activates anterior lobe of pituitary for secretion of FSH and LH which in turn leads to activation of thuriens[5,7].

In the treatment of Parkinson's disease L-dopa acts as precursor for the neurotransmitter dopamine[8-11]. Dopamine mediates L-dopa induced facilitation of sexual behavior in male rats.

Our previous study showed that *Mucuna cochinchinensis* (black seed variety) was found to have highest L-dopa content of 190.71 µg/ml, followed by *M. cochinchinensis* – white seed variety (96.22 µg/ml) and *M. pruriens* (77.68 µg/ml)[7].

In the present study, *M. cochinchinensis* black variety showed significant spermatogenetic activity which may be correlated with the presence of high amount of L-dopa as compared to other species of *Mucuna* studied.

Kapikachu (*Mucuna* sps) is being used for it's spermatogenetic (Vrishya) activity for centuries by Ayurvedic scholars, which is established through this study. This activity can be attributed to the presence of high levels of L-dopa. During this study it was also observed that spermatogenic effect of administration of L-dopa alone, as standard, to the animals could not produce the results on par with the aqueous extract of *Mucuna* sps treated animals. This could be due to the presence of zinc[12] and other phytoconstituents of the seeds[13].

Shukla *et al*.[2] had reported that on treatment with *M. pruriens* in infertile men there was a significant improvement in serum testosterone, luteinizing hormone, dopamine, adrenaline, and noradrenaline levels and reduced levels of follicle stimulating hormone (FSH) and prolactine hormone (PRL). Sperm count and motility were significantly recovered in infertile men. In the present study also similar observations were seen in rats treated with *Mucuna* extracts. *M. pruriens* seed powder rejuvenates the harmonic balance of male reproductive hormones in infertile men and reactivates the enzymatic activity of metabolic pathways and energy metabolism[14].

CONCLUSION

The data obtained in this study revealed that spermatogenesis and sperm parameters significantly changes during treatment with high dose of *Mucuna cochinchinensis* (Black seed) followed by *Mucuna pruriens* and *Mucuna cochichinensis* (White seed) in rats.

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REFERENCES

1. Vagbhata, Astanga Samgraha', edited by Atrideva Vaidya Pankara, Bansa Pathaka, Varanasi, (1975).

2. Shukla KK, Mahdi AA, Ahmad MK, Jaiswar SP, Shankwar SN, Tiwari SC. Mucuna pruriens Reduces Stress and Improves the Quality of Semen in Infertile Men . Evidence-Based Complementary and Alternative Medicine. 2010;7(1):137–44. Available from: http://dx.doi.org/10.1093/ecam/nem171.

3. Jadhav SR, Physiological study of Shukravaha Srotas and clinical study of kapikacchu Churna in Klaibya

with special Ref. to oligozoospermia (Thesis), PG Dept. Of Sharir kriya, NIA Jaipur. 2013; 141-145.

4. Jadhav SR, Dadhich OP, & Kothari P, KAPIKACCHU (Mucuna pruriens) - A Ayurvedic drug review, World J Pharm Sci. 2015; 3(10):1999-2003. http://www.wjpsonline.org/issue.php?id=MzM

5. Singh AP, Sarkar S, Tripathi M, Rajender S. Mucuna pruriens and Its Major Constituent L-DOPA Recover Spermatogenic Loss by Combating ROS, Loss of Mitochondrial Membrane Potential and Apoptosis. Vavvas D, editor. PLoS ONE. 2013 Jan 22;8(1):e54655. Available from: http://dx.doi.org/10.1371/journal.pone.0054655.

6. Khaki A, Fathiazad F, Nouri M, Khaki AA, Ozanci CC, Ghafari-Novin M, & Hamadeh M. The Effects of Ginger on Spermatogenesis and Sperm parameters of Rat. Iranian J. of Reproductive Med. 2009; 7(1):7–12. https://scholar.google.co.in/citations?view_op=view_citation&hl=en&user=eHppksIAAAAJ&citation_for_view=eHppksIAAAAJ:fbc8zXXH2BUC

7. Murthy S. N., Malgaonkar MM, Shirolkar AR, Pawar SD, Sangvikar S, & Kulkarni YR. A comparative assessment of pharmacologically active principles and antioxidant activity of commonly occurring Mucuna sps. in India. International Journal of Ayurveda and Pharma Research. 2015; 3(11):8-13. http://ijapr.in/index.php/ijapr/article/view/2/17

8. Daxenbichler ME, VanEtten CH, Hallinan EA, Earle FR, Barclay AS. Seeds as sources of L-Dopa. Journal of Medicinal Chemistry. 1971 May;14(5):463–5. Available from: http://dx.doi.org/10.1021/jm00287a030.

9. Anonymous (1973) British Pharmacopoeia, London.

10. Manyam BV. An alternative medicine treatment for Parkinson's disease: results of a multicenter clinical trial – HP-200 in Parkinson's disease study group. J. Alternat. Comp. Med. 1995; 1 : 249-255.

11. Longhi JG, Perez E, Lima JJ de, Cândido LMB. In vitro evaluation of Mucuna pruriens (L.) DC. antioxidant activity. Braz J Pharm Sci. 2011 Sep;47(3):535–44. Available from: http://dx.doi.org/10.1590/s1984-82502011000300011.

12. Cheah Y, Yang W. Functions of essential nutrition for high quality spermatogenesis. ABB. 2011;02(04):182–97. Available from: http://dx.doi.org/10.4236/abb.2011.24029.

13. Murthy SN, Sangvikar S, Malgaonkar MM, Sharma C & Kulkarni YR. In vitro physicochemical, phytochemical and fluroscence assessment of Mucuna sps. IOSR Journal of Biotechnology and Biochemistry. 2016; 2(2):1-10. http://iosrjournals.org/ iosr-jbb/papers/Vol2-ssue2/A0220110.pdf

14. Gupta A, Mahdi AA, Ahmad MK, Shukla KK, Bansal N, Jaiswer SP, et al. A proton NMR study of the effect of Mucuna pruriens on seminal plasma metabolites of infertile males. Journal of Pharmaceutical and Biomedical Analysis. 2011 Jul;55(5):1060–6. Available from: http://dx.doi.org/10.1016/j.jpba.2011.03.010.

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In vitro Physico-chemical, Phytochemical and Fluroscence Assessment of Mucuna sps.

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Abstract: *Mucuna is commonly called as "Kapikacchu', which is known to have various medicinal properties and being used in Ayurveda since ages. Medicinal plants have bioactive compounds which are used for curing various diseases. The present investigation is aimed to screen the various bioactive compounds present in seeds of Mucuna pruriens (L.) DC., Mucuna cochinchinensis (Lour.)A. Chev. black seeds variety and Mucuna cochinchinensis (Lour.) A. Chev. white seeds variety. In the present study, an attempt was made to analyse and evaluate the presence of various phytoconstituents as well as fluorescent characteristics of seeds of Mucuna sps. Along with this, during the research work, physicochemical parameters were also determined. Qualitative phytochemical analysis of the aqueous and methanolic extracts of seeds showed the presence of various phytoconstituents i.e. carbohydrates, reducing sugar, hexose sugar, proteins, amino acids, steroids, saponins, flavonoids, alkaloids, tannins and phenols. Qualitative analysis of the aqueous extract was carried out. It was observed that all the extracts show more important chemical constituents for various pharmacological activities. In fluorescent analysis characteristic colours were observed with different chemical reagents in seed powder extracts under visible and UV light (short and long wavelength). The determination of these characters will aid future investigators in their Pharmacological analysis of this species. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases.*

Keywords: *Kapikacchu, Fluorescence, Physicochemical analysis, Mucuna pruriens (L.) DC., Mucuna cochinchinensis (Lour.)A. Chev.*

I. Introduction

Kapikacchu (Mucuna belongs to the family Fabaceae) is an ingredient of several commercial preparations that are being used as an aphrodisiac, in male sexual disorders. Seeds are astringent, laxative, anthelminitc, alexipharmic, hypotensive, spasmodic, hypocholestrolemic, 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 [1], treatment of pain and numbness of joints, and irregular menstruation [2].

The genus Mucuna belongs to the family Fabaceae (Leguminoceae) 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 [3]. 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 [4, 5]. Almost all the species are reported to be used in the treatment of Parkinson's disease [6].

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 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 commonly occurring Mucuna sps in India.

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]. It is reported to be native of China and Eastern India [9]. M. pruriens is a wild plant and it's every part is full of medicinal value. It's most important parts are seeds and roots which are good source of giving vital energies. Seeds are excellent source of L-DOPA (lavodopa 3,4- dihydroxyphenyl alanine) which is precursor of dopamine a neurotransmitter [10] used in the treatment of Parkinson's disease [11,12].

Mucuna cochinchinensis (Lour.)A. Chev. 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 [13]. The seeds of M. cochienchinensis contains carbohydrate 55.8%, protein 27.5% and fat 3.6%. The fruits yield 0.96% L-dopa [14].

The present study was aimed to assess and compare the physicochemical and phytochemical (qualitative as well as quantitative) parameters of Mucuna pruriens (L.) DC., Mucuna cochinchinensis (Lour.)A. Chev. black seeds variety and Mucuna cochinchinensis (Lour.) A. Chev. white seeds variety.

II. Materials and Methods

1. Collection and Processing

Seeds of Mucuna pruriens (L.) DC., Mucuna cochinchinensis (Lour.)A. Chev. black seeds variety and Mucuna cochinchinensis (Lour.) A. Chev. white seeds variety were collected from Medicinal Plant Garden of National Research Institute of Basic Ayurvedic Sciences, Kothrud, Pune, India during the month of January - February, 2013. Samples were taxonomically identified in the institute and were then deposited in the herbarium section for future reference. Samples were taxonomically authenticated by Plant Sciences Division of Agharkar Research Institute, Pune with voucher numbers S-159 (Mucuna cochinchinensis (Lour.)A. Chev. black seeds variety), S-161 (Mucuna cochinchinensis (Lour.)A. Chev. white seeds variety), and S-162 (Mucuna pruriens (L.) DC.).

2. Physicochemical Parameters

Physicochemical parameters like Soluble extractive values of dried seeds powder in Absolute Alcohol, Water, Methanol, Acetone, Ethyl acetate, Petroleum ether, Benzene, Chloroform, Carbon tetrachloride, Hydro-alcoholic; Total ash; Acid insoluble ash; Water soluble ash, Loss on drying (LOD); Foreign matter; Swelling factor, Specific gravity and pH value were determined using the standard protocols and guidelines.[15-17]

3. Extracts Preparation for Phytochemical analysis

Extraction of dried plant material was carried out using a method described by Harborne (1973) with minor modifications. The leaves were thoroughly washed under tap water followed by washing with distilled water.[18] Collected seeds as well as washed leaves were shade dried and powdered. The powdered leaves and seeds were kept overnight in different solvents systems like water (aqueous), methanol and hydro-alcohol. Extracts were filtered using Whatmann no. 42 (125mm) filter paper. The extracts were evaporated to reduce the volume to half (approximately) on a water-bath for 1-2 hrs. The plant extracts were cooled and then a part of the extract was used directly for qualitative determination of phytochemical parameters and remaining extract was subjected to lyophilization which was further taken to evaluate the quantitative parameters.

4. Qualitative Phytochemical Analysis

The following tests were carried out to determine the presence of various active phyto-constituents.

Carbohydrates: To 2-3 ml extract, few drops of Molisch reagent was added, mixed well and conc. H_2SO_4 was added from the sides of the test tube, violet ring formation at the junction of two liquids indicated the presence of carbohydrates.[19]

Sugars: Presence of Reducing sugar, Pentose sugar and Hexose sugar were determined using the Fehling's, Aniline acetate and Cobalt Chloride test respectively as described by Sadasivam and Manickam (1996) and Kokate (1996) [20,21].

Amino acids: Ninhydrin test was conducted for amino acids in general and presence of cysteine was checked by adding 40% NaOH and 10% lead acetate solution to extract. Appearance of black lead sulphate precipitate after boiling confirmed the test [20].

Proteins: Presence of proteins was determined by Biuret test, Million's test, Xanthoprotein test, heat test, Lead acetate test and Ammonium sulphate test [20].

Steroids: Salkowski test and Libermann-Buchard test were conducted for assessing the presence of steroids. While performing Libermann-Buchard test to 2 ml extract with chloroform.1-2 ml aceteic unhydride few drops cons. H_2SO_4 was added from the side of test tube. Steroids were indicated by reddish brown coloured ring at the junction of two layers [22].

Sakowski test: To 2 ml of extract 2 ml chloroform and 2 ml of conc. H_2SO_4 was added and mixed well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence [21].

Glycosides: To 2 ml extract glacial acetic acid, few drops of 5% $FeCl_3$ and conc. H_2SO_4 were added. Reddish brown colour at the junction of two liquid layers and upper layer appears bluish green indicating the presence of glycosides.[23,24] Presence of Cardiac Glycosides, Anthroquinone glycosides [25,26] and Coumarin glycosides[27] were determined using the protocols described by Sadasivam and Manickam (1996) and Kokate (1996) [20,21].

Saponins: Foam and lead acetate tests were carried out for indicating the presence of saponins. The layer of foam formation on vigorous shaking of extract and distilled water confirmed the presence of saponins in foam test and formation of white precipitate on the addition of lead acetate indicated the positive saponin test [28, 29].

Flavonoids: Shinoda test, Lead acetate test and alkaline reagent tests were conducted for the flavonoid determination [21,28-30].
Shinoda Test: An alcoholic extract of plant material was treated with magnesium (dust) and conc. HCl. The appearance of Crimson red colour indicated the presence of flavonoids.
Lead Acetate test: To the small quantity of extract, lead acetate solution was added then yellow or reddish brown coloured precipitate determined the presence of flavonoids.
Alkaline Reagent test: The test solution was treated with sodium hydroxide which showed yellow coloration, indicating the presence of flavonoids.
Alkaloids: For the confirmation of alkaloids three tests were conducted [20, 21, 28].
Dragendroff's test confirmed the presence with formation of orange brown precipitate on addition of few drops of dragendroff's reagent onto the extract.
Mayer's test: Few drops of the Mayer's reagent was when added onto the extract, white - pale yellow precipitate was seen which indicated the presence of alkaloids.
Wagner's test**:** Extract was acidified with 1.5 % v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicated the presence of alkaloids.
Tannins: Lead acetate test was performed for confirming the presence of tannins in the extract. To an aqueous solution of dried plant material, 5% lead acetate solution was added; appearance of white precipitate indicated the presence of tannins [20, 28, 31].
Mucilage: Mucilage test was conducted as per the procols described in Kokate (1996). Powered drug material showed red color with ruthenium red [21].
Phenol: For the purpose of analysing the presence of phenol $FeCl_3$ test and Folin-ciocaltaue test was carried out [21, 32].
$FeCl_3$ Test: Small amount of extract of plant material was treated with neutral $FeCl_3$. Formation of blue or green colour indicated the presence of phenol [31].
Folin-ciocaltaue reagent test: When the extract was treated with a little residue of ammonia solution, a blue or blue to gray colour is formed indicating the phenols are present.
Starch: Iodine test was conducted for confirming the presence of starch in the extract. To 3 ml of extract few drops of dilute iodine solution was added and mixed. Appearance of blue colour which disappears on boiling and reappears on cooling indicated the presence of starch [20, 21].

5. Quantitative Phytochemical Analysis

For Quantification of various phytochemical constituents' standard procedures for estimation were performed. The parameters like Carbohydrates [16]; Flavanoids [33-35]; Tannins [35]; Proteins [36]; Glycosides [23,36]; Lipids [37] and Total Phenol Content[32,38] were quantified for all the Mucuna sps under study.

6. Fluorescence analysis of Powder

Fluorescence analysis of the powder was carried out as described by Nanna et al., 2013 and Birajdar et al., 2014 [39,40]. Seed and leaf powder were taken in different tubes. To each of these a fixed volume of different organic solvents like distilled water, ethanol, benzene, chloroform, methanol, glacial acetic acid, sulphuric acid, nitric acid, hydrochloric acid and ammonia were added. Then all the tubes were observed under the visible and UV light after shaking for the visualization of characteristic colour reaction were compared with a standard colour chart and colours were noted [41].

III. Results

This study revealed the presence of physicochemicals and phytochemicals considered as active medicinal chemical constituents. The physicochemical constituents of Mucuna sps are presented in Table 1. Total ash was highest in M. pruriens (3.88%) Physicochemical parameter analysis is studied for understanding the nutritional value, quality and microbiological stability of the drug. Swelling factor was not found to be present in M. pruriens. Table 2, represents the specific gravity of Mucuna sps extract with different solvents. These results show that all the species almost same specific gravity in each solvent.

Extractive values for Mucuna sps in different solvents were determined and depicted in Table 3. The comparison between different solvents and Mucuna sps showed that seed powder has significant extractive values in water, methanol and hydro-alcohol extracts.

In qualitative analysis of Mucuna sps it was found that, methanolic and water extracts showed the presence of alkaloids (Wagner's test) and phenols. When protein presence was checked using lead acetate test only methanolic extract gave positive detection. Hexose sugar was found only in methanolic extract of M. pruriens. Carbohydrates, reducing sugar, saponins, flavonoids (lead acetate test), alkaloids (Mayer's test) and starch was detected when water extract of the samples were analyzed. Volatile oil, organic acids, mucilage glycosides, pentose sugar and Cysteine amino acid was not detected in any of the sample and extract (Table 4).

The screening and quantitative analysis using water extract of Mucuna sps for the carbohydrates, flavonoids, tannins, lipids, proteins, total phenolic content and glycosides is presented and expressed in Table 5 followed by respective graphs (Fig. 1-7).

The results of the quantitative phytochemical analysis showed that the seeds M. cochienchinensis white seed variety are rich in carbohydrates, flavonoids, and tannins whereas M. cochienchinensis black seed variety showed significant quantity of proteins and total phenolic contents. In the quantification of lipid content and glycosides (though the amount was significantly low in all the sps under study) were present in maximum amount in M. pruriens.

The results of fluorescence analysis of Mucuna seeds with different solvents are expressed in Table 6. Fluorescence study is an essential and required parameter for first line standardization of crude drug. In this analysis most of the reactions resulted in colourless reaction mixture.

IV. Tables

Table 1: Physicochemical constituents of Mucuna sps

Sr. No.	Parameters	Mucuna pruriens (L.) DC.	Mucuna cochinchinensis (Lour.) A. Chev. (White Seed)	Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed)
1	pH	7.40	7.65	7.43
2	Loss on Drying (LOD)	5.56 %	10.15 %	9.29 %
3	Total Ash	3.88 %	3.42 %	3.53 %
4	Acid Insoluble Ash	0.09 %	0.04 %	0.10 %
5	Water soluble Ash	0.14 %	0.05 %	0.07 %
6	Swelling factor	0.0 ml	1.0 ml	1.0 ml

Table 2: Specific gravity of Mucuna species extract with different solvents

Sr. No.	Solvents	Mucuna pruriens (L.) DC.	Mucuna cochinchinensis (Lour.) A. Chev. (White Seed)	Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed)
1	Water	0.9831	0.9698	0.9739
2	Methanol	0.7801	0.5151	0.7669
3	Ethanol	0.7773	0.8160	0..7655
4	Acetone	0.7719	0.7935	0.7586
5	Ethyl acetate	0.8851	0.9001	0.8700
6	Petroleum ether	0.6518	0.6607	0.6452
7	Benzene	0.8570	0.8738	0.8574
8	Chloroform	1.4539	1.4454	1.4266
9	Carbon tetra chloride	1.5526	1.5521	1.5176

Table 3: Determination of Extractive values

Sr. No.	Extract	Mucuna pruriens (L.) DC. (in %)	Mucuna cochinchinensis (Lour.) A. Chev. (White Seed) (in %)	Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed) (in %)
1	Absolute alcohol	9.61	13.46	14.08
2	Water	29.00	21.89	24.82
3	Methanol	19.05	12.32	15.83
4	Acetone	7.41	6.07	10.74
5	Ethyl acetate	5.02	5.17	9.60
6	Petroleum ether	5.34	5.17	11.41
7	Benzene	5.84	5.63	11.05
8	Chloroform	8.20	8.22	15.05
9	Carbon tetra chloride	8.20	7.61	11.12
10	Hydroalcoholic (1:1)	38.64	38.13	38.05

Table 4: Qualitative analysis for Phytochemicals

Sr. No.	Test	Test Method	Mucuna pruriens (L.) DC.		Mucuna cochinchinensis (Lour.) A. Chev. (White Seed)		Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed)	
			ME	WE	ME	WE	ME	WE
1	Carbohydrates	Molish's test	-	+	-	+	-	+
2	Reducing sugar	Fehling's test	-	+	-	+	-	+
3	Pentose sugar	Phloroglucinol reag. Test	-	-	-	-	-	-
4	Hexose sugar	Tollen's Phloroglucinol test	+	-	-	-	-	-
5	Proteins	Biuret test	-	-	-	-	-	-
		Millon's test	-	-	-	-	-	-
		Xanthoprotein test	-	-	-	-	-	-
		Heat test	-	-	-	-	-	-
		5% Lead acetate	+	-	+	-	+	-
		5% Ammonium sulphate	-	+	-	-	-	-
6	Amino acid	a)Ninhydrin test	+	+	+	+	+	+
		b)Test for cysteine	-	-	-	-	-	-
7	Steroids	a)Libermann-Burchard Test	+	+	-	-	-	-
		b)Salkowski reaction	+	-	-	-	-	-
8	Glycoside	General test	-	-	-	-	-	-
9	Cardiac Glycosides	Legal test	-	-	-	-	-	-
		b)Keller-Killiani test	-	-	-	-	-	-
10	Anthroquinone glycoside	Borntrager's test	-	-	-	-	-	-
11	Cyanogenetic glycoside	a)sodium picrate test	-	-	-	-	-	-
		b)mercurous nitate test	-	-	-	-	-	-
12	Coumarin glycoside	a)No aromatic odor	-	-	-	-	-	-
		b)No green fluorescence	-	-	-	-	-	-
13	Saponins	a)Foam test	-	+	-	+	-	+
		b)Lead acetate test	-	-	-	-	-	-
14	Flavonoids	a)Shinoda test	-	-	-	-	-	-
		b)Lead acetate test	-	+	+	-	+	-
		c)Alkaline reagent test	-	-	-	-	-	-
15	Alkaloids	a)Dragendorff's test	-	-	-	-	-	-
		b)Mayer's test	-	+	+	-	+	-
		c)Wagner's test	+	+	+	+	+	+
16	Tannins	Lead acetate test	-	-	+	-	+	-
17	Mucilage		-	-	-	-	-	-
18	Phenol	a)$Fecl_3$ test	+	+	+	+	+	+
		b)Folin-ciocaltaue reagent	+	+	+	+	+	+
19	Starch	Iodine test	-	+	-	+	-	+
20	Organic acids	Calcium Chloride test	-	-	-	-	-	-
21	Volatile Oil		-		-		-	

ME: Methanolic extract; WE: Water extract; (+): Present; (-): Not present.

Table 5: Quantitative Phytochemical analysis

Sr. No.	Phyto-constituents	Mucuna pruriens (L.) DC. (µg/ml)	Mucuna cochinchinensis (Lour.) A. Chev. (White Seed) (µg/ml)	Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed) (µg/ml)
1	Carbohydrates	57.4	146.6	275.8
2	Flavonoids	42.18	35.6	65.0
3	Tannins	203.6	306.2	576.7
4	Glycosides	56.28	10.16	32.8
5	Proteins	110.41	314	270.27
6	Total Phenolic content	10.42	80.68	40.00
7	Lipids	39.2	9.12	10.41

Fig. 1: Graphical representation of quantitation of Carbohydrates

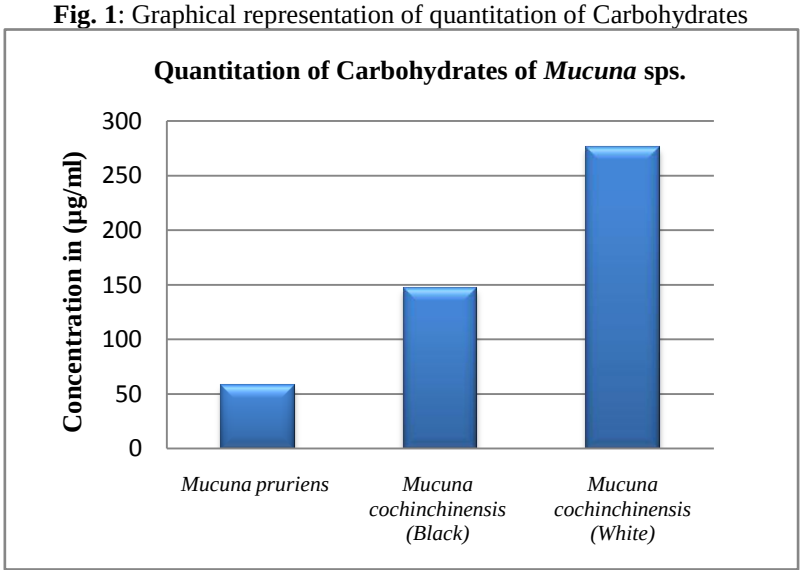


Fig. 2: Graphical representation of quantitation of Flavonoids

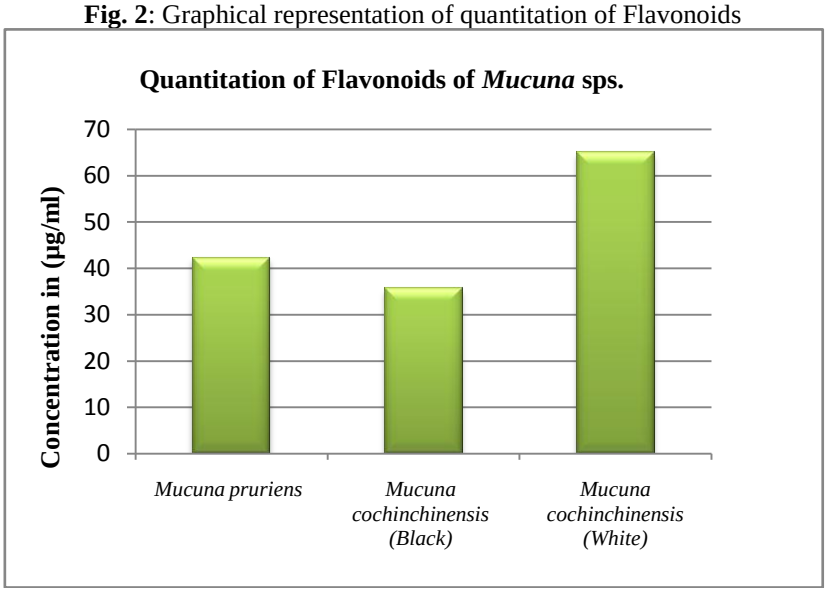


Fig. 3: Graphical representation of quantitation of Tannins

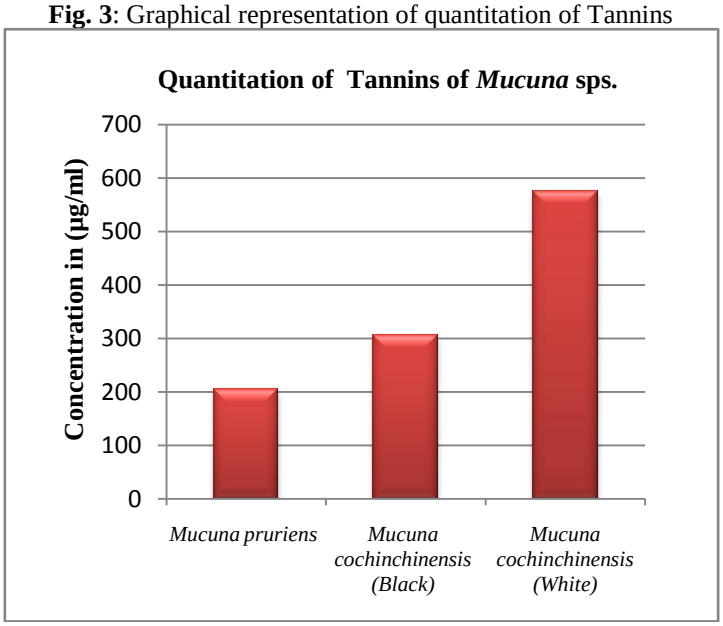


Fig. 4: Graphical representation of quantitation of Lipids

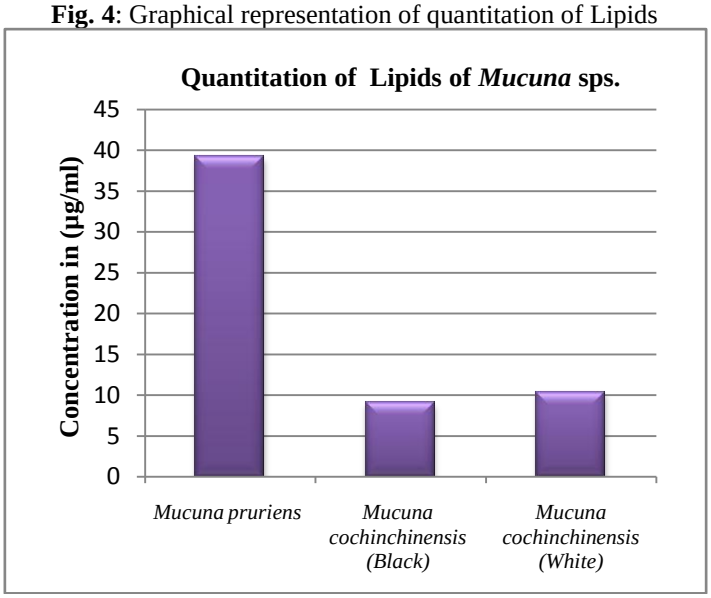


Fig. 5: Graphical representation of quantitation of Proteins

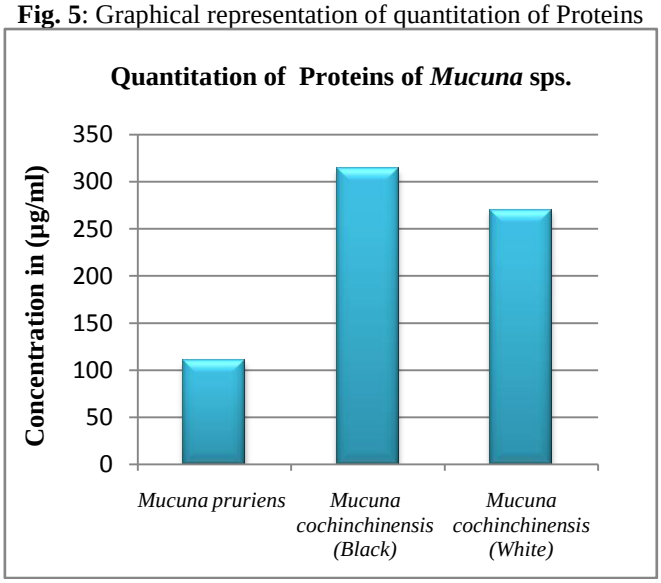


Fig. 6: Graphical representation of quantitation of Phenolic content

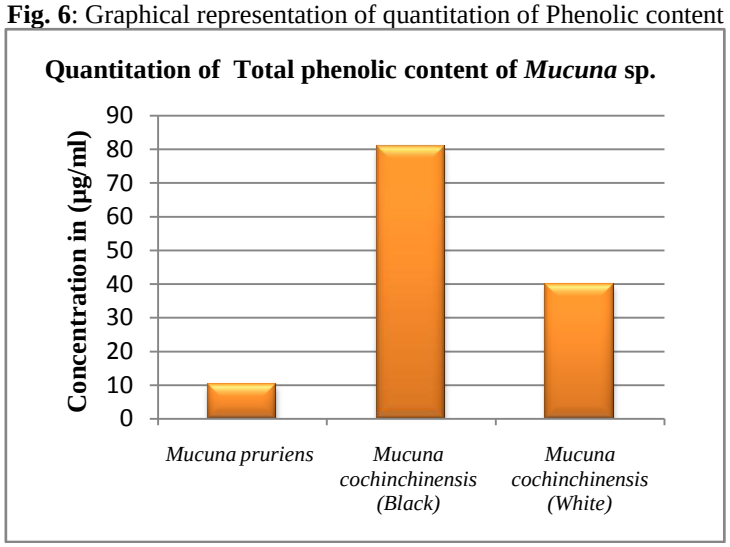


Fig. 7: Graphical representation of quantitation of Glycosides

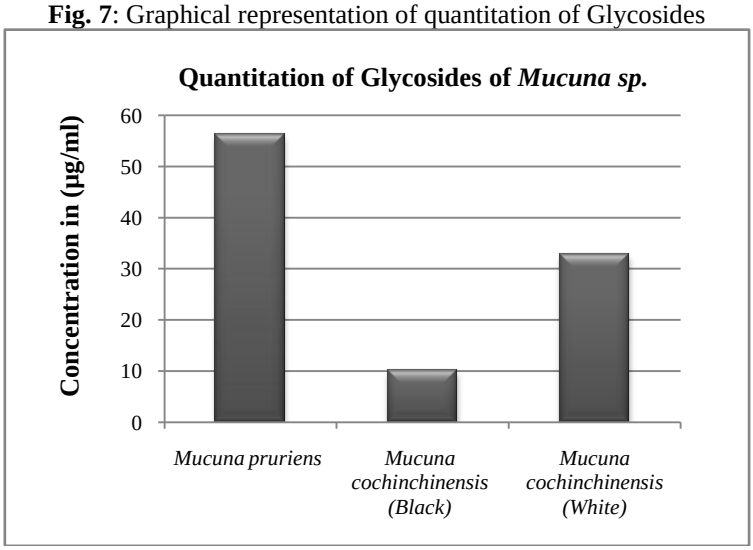


Table 6: Fluorescence of Mucuna species with different solvents

Sr. No.	Solvents	Mucuna pruriens (L.) DC.			Mucuna cochinchinensis (Lour.) A. Chev. (White Seed)			Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed)		
		Day light	Short UV	Long UV	Day light	Short UV	Long UV	Day light	Short UV	Long UV
1	Water	Light brown	Light umber	Light purple	Dark brown	Dark blackish green	Dark purple	Light brown	Chestnut	Dark purple
2	Acetone	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless
3	Benzene	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless
4	Chloroform	Colourless	Colourless	Colourless	Light yellow	Colourless	Colourless	Colourless	Buff	Colourless
5	Carbon tetra chloride	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Very Light brown	Light yellow	Colourless
6	Ethanol	Light yellow	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless
7	Ethyl acetate	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless
8	Methanol	Light yellow	Colourless	Colourless	Light pale yellow	Light pale yellow	Colourless	Light yellow	Light yellow	Colourless
9	Petroleum ether	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless

V. Conclusion

Phytochemical investigation of seed extracts showed the presence of alkaloids, phenols, carbohydrates, reducing sugar, saponins, flavanoids, starch, proteins and hexose sugar at least in one of the extract. These results are supportive with other studies perfomed with Mucuna sps, where same secondary metabolites were found [42]. Qualitative phytochemical screening is an essential step towards discovery of new drug as it provides the information regarding the presence of primary and secondary metabolites in the plant extract. The methods employed to isolate active substance are termed as extractive method. Crude extracts obtained from such extraction can be qualitatively tested to ascertain the presence of different types of components.

Volatile oil, organic acids, mucilage, glycosides, pentose sugar and cystine amino acid was not positive in any of the extract and Mucuna sps under study, indicating the absence of respective phytochemicals whereas, steroid was found to be present only in M. pruriens in less quantity (as the reactions were not very prominent) suggesting that the plant does not possess those phytochemical in abundance. Qualitative tests are used to detect the presence of functional groups, which play very important role in the expression of biological activity. These tests confirm the presence of certain types of phyto-constituents in the sample.

Correlation between the phyto-constituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic disease [43]. Owing to the significance in the above context, such preliminary phytochemical screening of plants is the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy. Antitumor, anti inflammatory and antimicrobial properties are due to the presence of alkaloids [44]. Antiparasitic, antibacterial and antifungal activities are due to the presence of flavonoids [45, 46]. Tannins are used as tanning agents as they possess astringent, antioxidant and antimicrobial activities. Bactericidal and fungicidal properties are due to the presence of tannins [47-48].

In fluorescence the fluorescent light is always of greater wavelength than the exciting light. Light rich in short wavelengths is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substances which do not visibly fluoresce in daylight [49, 50].

The research work was carried out on Mucuna sps. seeds, which showed the presence of various phytochemical constituents and bioactive compounds in all the extracts in varying range and quantity. The quality and quantity of phytochemicals is based on the selection of solvent system and procedure selected for extraction. The selected plant is the source of secondary metabolites. The phytochemical constituents of Mucuna.pruriens and Mucuna cochinchinensis would be helpful in treating many diseases and the fluorescent analysis of powdered drug can play a remarkable role in the determination of quality and purity of the raw drug. These types of studies have vital role because of the commercial and research interest.

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References

[1]. Standley, P.C. and Steyermark, J.A. (1946). Flora of Guatemala. Fieldian: Botany Vol 24 Part V.
[2]. Ding, Y., Kinjo, J., Yang, C. and Nohara, T. (1991). Triterpenes from Mucuna birdwoddiana. Phytochemistry 30(11): 3703-3707.
[3]. Eilittä, M., Bressani, R., Carew, L.B., Carsky, R.J., Flores, M., Gilbert, R., Huyck, L., St-Laurent, L. and Szabo, N.J. (2002). Mucuna as a food and feed crop: an overview. In: Mucuna as a Food and Feed: Current Uses and the Way Forward. Ed. By Flores M, Eilittä M, Myhrman R, Carew L, Carsky R, Workshop held April 26-29, 2000 in Tegucigalpa, Honduras. CIDICCO Honduras. pp. 18-46.
[4]. Janardhanan K. and Lakshmanan K.K. (1985). Studies on the pulse, Mucuna utilis. Chemical composition and anti-nutritional factors. J. Food Sci. Tech. 22: 369-37.
[5]. Mohan, V.R. and Janardhanan, K. (1993). Studies on the Indian tribal pulses. In proceedings of the national seminar on biodiversity: strategies for conservation and future challenges. Department of Botany. Bharathiar University. Ed. by Udaiyan K, Janardhanan K, Manian S, Reddy VRKP. pp. 116-125.
[6]. Manyam, B.V. (1995). An alternative medicine treatment for Parkinson's disease: results of a multicenter clinical trial – HP-200 in Parkinson's disease study group. J. Alternat. Comp. Med. **1**: 249-255.
[7]. Sastry, C.S.T. and Kavathekar, Y.Y. (1990). Plants for reclamation of wastelands. Publications and Information Directorate, New Delhi. p.317-318.
[8]. Singh, U., Wadhwani, A.M. and Johri, B.M. (1996). Dictionary of economic plants in India. Indian Council of Agricultural Research, New Delhi. p. 45-46.
[9]. Wilmot Dear C.M. (1987). A revision of Mucuna (Leguminosae - Phaseolae) in the Indian Subcontinent and Burma. Kew Bull., **42(1)**: 23–46.
[10]. Daxenbichler, M.E., Van Etten, C.H., Hallman, E.A., Earle, F.R. and Barclay, A.S. (1971). Seeds as sources of L-DOPA. J. Med. Chem. **14**: 463.
[11]. British Pharmacopoeia. (1973), London.
[12]. Longhi, J.G., Perez, E., Jose de Lima, J. and Candido L.M.B. (2011). In vitro evaluation of Mucuna pruriens (L.) DC. antioxidant activity. Brazilian Journal of Pharmaceutical Sciences, **47(3)**: 535-544.
[13]. Anonymous. (2005). The Wealth of India Raw Materials, NISCAIR Publishing New Delhi, L-M; **VI**: 439-444.
[14]. Anonymous. (2006). The Wealth of India- Raw Materials. First Suppl Ser. NISCAIR; 166-167.

[15]. Anonymous. Quality Control Methods for Medicinal Plants Materials. Geneva: World Health Organization: 1998, pp. 6-22.
[16]. Khandelwal KR. Practical Pharmacognosy Technique and Experimental. Pune: Nirali Prakashan; 2001, pp. 149-156.
[17]. Subramaniam P, Sunil Kumar. Physicochemical profile of some colouring plants used in Homeopathy. Ancient Science of life, 2001; 21(2): 1-8.
[18]. Harborne JB. Phytochemical methods. Chapman and Hall, London. 1973
[19]. Krishnadevi S, Theymoli B and Sadasivam S. Phenol Sulphuric acid method. Food Chem. 1984. 15: 229
[20]. Sadasivam S, Manickam A. Biochemical methods. New age International (P) Limited, Publishers, New Delhi, 1996.
[21]. Kokate CK. Practical Pharmacognosy. 4th ed. Reprint. Delhi: Vallabh Prakashan; 1996, pp. 10-321.
[22]. Wall ME, Krider MM, Krewson CF, Eddy CR, Willaman JJ, Correll DS, Gentry HS. Steroidal sapogenins – Survey of plants for steroidal sapogenins and othe constituents VII, Jour. Amer. Pharm. Ass., Sci Ed., 1954, 43: 1-7.
[23]. Trease GE, Evans WC. Pharmacognosy. 11th edn; Brailliar Tiridel Can. Macmillian Publishers 1989.
[24]. Harborne JB. Phytochemical Methods—A Guide to Modern Techniques of Plant Analysis. 1stedn: Chapman and Hall; London, UK. 1998.
[25]. Harbourne JB. Phytochemical Methods: A Guide to Modern Technique of Plant Analysis, 2nd ed. Chapman and Hall, London. 1984; 282.
[26]. Sofowora A. Phytochemical screening of medicinal plants and traditional medicine in Africa 2nd Edition Spectrum Books Limited, Nigeria, 1993, 150-156.
[27]. El-Tawil BDH. Chemical constituents of indigenous plants used in Native Medicines of Saudi Arabia, II. Arab Gulf J Sci Res, 1983; (12): 395 -410
[28]. Siddiqui AA, Ali M. Practical Pharmaceutical Chemistry. 1st edn; CBS Publishers and distributors, New Delhi, 1997; 126-131.
[29]. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. Internationale Pharmaceutica Sciencia, 2011; 1: 98-106
[30]. Brain KR, Turner TD. The practical evaluation of phytochemicals. Wright Science Technical:Bristol: 1975
[31]. Kokate CK. A Textbook for Practical Pharmacognosy. 5th Ed edn. 2005.
[32]. Gibbs RD. Chemotaxonomy of Flowering Plants. Vol.1, McGill Queen's University Press Montreal and London. 1974.
[33]. Peach K., Tracey MV. Modern methods of plant analysis. Vol.3, Springer Verlag, Berlin. 1956.
[34]. Khatiwora E, Adsul VB, Kulkarni MK, Deshpande NR, Kashalkar RV. Spectroscopic determination of total phenol and flavonoid contents of Ipomoea carnea. International Journal of ChemTech Research, 2010; 2(3), 1698-1701.
[35]. Trease GE and Evans WC. Pharmacognosy. 17th edn; Bahive Tinal, London. 1985, PP 149.
[36]. Lowry OH, Rosebrough NR, Lewis Farr A, Randall RJ. Protein measurement with the Folin Phenol Reagent, J. Biol. Chem, 1951; 193: 265-275.
[37]. Ganai BA, Masood A and Zargar MA. Experimental Biochemistry, 1st ed., Valley Publications, J&K, 2005; 148-149.
[38]. Mallick CP, Singh MB. Plant enzymology and Histoenzymology. Kalyani publishers, New Delhi 1980, 286.
[39]. Nanna RS, Banala M, Pamulaparthi A, Kurra A, Kagithoju S. Evaluation of Phytochemicals and Fluorescent Analysis of Seed and Leaf Extracts of Cajanus cajan L. Int. J. Pharm. Sci. Rev. Res., 2013; 22(1): 11-18.
[40]. Birajdar VV, Mhase AG, Gurav AM, Murthy SN. Preliminary pharmacognostic and phytochemical standardization of Dhataki [Woodfordia fruticosa (L.) Kurz.] leaves. Ayu 2014; 35:309-15.
[41]. Chase CR, Pratt RJ. Fluorescence of powdered vegetables drugs. Indian Journal of Experimental Biology, 1949; 33(6): 428-432.
[42]. Manalisha D and Chandra KJ. 2012. Priliminary phytochemical analysis and acute oral toxicity study of Mucuna pruriens L. in Albino mice. International Research journal of Pharmacy, 3(2): 181-183.
[43]. Pandey P, Mehta R, Upadhyay R. Physico-chemical and preliminary phytochemical screening of Psoralea corylifolia. Arch Appl Sci Res, 2013; 5:261-265.
[44]. Castilhos TS, Giordani RB, Henriques AT, Menezes FS, Zuanazzi JA. In vitro evaluation of the antioxidant, anti-inflammatory and antimicrobial activities of the montanine alkaloid. Braz J Pharmacogn, 2007; 17: 209–214.
[45]. Simões CM, Schenkel EP, Gosmann G, Mello JC, Mentz LA, Petrovick PR, Porto Alegre/ Florianópolis, Ed. Universidade/ UFRGS/ Ed. da UFSC, 4, Farmacognosia: Da planta ao medicamento. 2002.
[46]. Sen G, Mandal S, Saha Roy S, Mukhopadhyay S, Biswas T. Therapeutic use of quercetine in the control of infection and anemia associated with visceral leishmaniasis, Free Radic Biol Med, 2005; 38: 1257–1264.
[47]. Ho KY, Tsai CC, Huang JS, Chen CP, Lin TC, Lin CC. Antimicrobial activity of tannin components from Vaccinium viti-sidaea L. J Pharm Pharmacol, 2001; 53: 187– 191.
[48]. Killedar SG, More HN, Estimation of tannins in different parts of Memecylon umbellatum Burm, J Phar Res, 2010; 3(3): 554-556.
[49]. Arya V, Gupta R, Gupta VK. Journal of Chemical and Pharmaceutical Research, 2011; 3(3), 447-456
[50]. Kumar D, Kumar K, Kumar S, Kumar T, Kumar A, Prakash O. Asian Pacific Journal of Tropical Biomedicine, 2012; 169-175.

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Molecular Analysis of *Mucuna pruriens* (L.) DC. and *Mucuna cochinchinensis* (Lour.) A. Chev. Using RAPD Markers Helps to Understand Genetic Variations

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Abstract: *Fifteen species of Mucuna species are reported so far from India which are being used in the name of Kapikacchu by the ayurvedic practitioners since ancient times. Kapikacchu is an ingredient of several commercial preparations that are being used as an aphrodisiac in male sexual disorders. The samples of Mucuna pruriens (L.) DC. and Mucuna cochinchinensis (Lour.) A. Chev. were authenticated, analyzed and compared to illustrate the phylogenetic relationships between them. In this study, a set of 25 plants RAPD universal primers (RPI 1 – RPI 25) were used for genomic DNA amplification. The amplicons were compared and the phylogenetic tree was drawn with the help of bioinformatic analysis tool UPGMA (Unweighted Pair Group Method with Arithmetic Mean) by applying Jaccard's distance method. The Jaccard's similarity coefficient values suggested good variability in these two Mucuna sps. 107 RAPD fragments were obtained using twenty five primers. The RAPD PCR (Random Amplification of Polymorphic DNA- Polymerase chain reaction) amplification resulted in different successive polymorphic banding patterns with primers in both the plants that indicate significant genetic variations between the species. The creation of some baseline data is an important approach to study the genetic markers of Mucuna species as the information on the genetic diversity of Mucuna species is inadequate.*

Keywords*: Mucuna pruriens; Velvet bean; Mucuna cochinchinensis; Lyon bean; RAPD profiling; Genetic divergence; Molecular polymorphism*

I. Introduction

The genus *Mucuna* belongs to the family Fabaceae (Leguminoceae) and includes about 150 species of annual and perennial legumes of tropical distribution. India is one of the natural centers of origin of the *Mucuna* in the world (Eilittä *et al*., 2002). To date fifteen species of *Mucuna* species are reported from India. In addition to their traditional medicinal use many species of the genus offer an excellent source as cover crop and green manure (Janardhanan and Lakshmanan, 1985; Mohan and Janardhanan, 1993). 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 (Manyam, 1995). In addition, *Mucuna* is also traditionally used in various other applications like, dye (Standley and Steyermark, 1946), treatment of pain and numbness of joints, and irregular menstruation (Ding *et al*., 1991).

Mucuna pruriens Bak. 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 (Sastry and Kavathekar 1990; Singh *et al*., 1996). It is reported to be native of China and Eastern India (Wilmot Dear, 1987). M. pruriens is a wild plant and it's every part is full of medicinal value. It's most important parts are seeds and roots which are good source of giving vital energies. Seeds are excellent source of L-DOPA (lavodopa 3,4- dihydroxyphenyl alanine) which is precursor of dopamine a neurotransmitter (Daxenbichler *et al*.,1971) used in the treatment of Parkinson's disease (British pharmacopoeia, 1973; Longhi *et al*., 2011). The seed of *M. cochienchinensis* contains carbohydrate 55.8%, protein 27.5% and fat 3.6%. The fruits yield 0.96% L-dopa (Anonymous, 2006)**.**

Mucuna cochinchinensis (Lour.) A. Chev., 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 (Anonymous, 2005).

Randomly Amplified Polymorphic DNA (RAPD) is convenient technique to analyse the genetic variation (Bhat *et al*., 1999). It has been extensively used for molecular fingerprinting and population diversity analysis (Albert *et al*., 1997, Saini *et al*., 2010). Availability of reliable polymorphic markers, often limits the accurate estimation of genetic variation among individuals or different populations.

In the present study, we have applied a DNA fingerprinting technique, random amplified polymorphic DNA (RAPD), to differentiate the three accessions of *Mucuna* as well as for genetic characterization of the selected accession.

II. Material And Methods

Collection of Plant Materials:

The leaves of *Mucuna pruriens* (L.) DC. and *Mucuna cochinchinensis* (Lour.) A. Chev. were collected from the Medicinal plants garden of National Research Ayurvedic Institute of Basic Ayurvedic Sciences, Pune. The plant materials were authenticated by Mrs. A. G. Mhase, Herbarium Assistant, and the specimens were preserved in the herbarium for reference.

DNA Extraction:

For RAPD analysis, healthy and non-infected leaves were collected from mother plant. The leaves samples were grounded in liquid nitrogen to a fine powder and were kept at -20°C until use**.** The total genomic DNA extraction from the leaves of both the plants was done by using 3B Black Biotech Biotools kit with approximately 100-120 mg of powdered sample of each. The DNA of both plants was then quantified using biophotometer (eppendorf) as well as standardized against known concentrations of DNA marker ladder (Promega, USA) on 1% agarose gels. DNA was then kept at 4°C until further use.

RAPD-PCR:

The protocol for polymerase chain reaction (PCR) was optimized by varying the concentration of template, *Taq* DNA polymerase and $MgCl_2$. Polymerase chain reaction (PCR) amplifications were performed in reaction mixtures containing 10 ng template DNA, 0.2 µM primer, 100 µM dNTP´s, 10 mM PCR buffer, 1.5 mM $MgCl_2$, and 1 unit *Taq*-polymerase in a total volume of 20 µl.

Amplifications were carried out in a thermo-cycler (Peltier P25+, Cyber lab) with the following program: initial denaturation step of 3 min at 94°C followed by 35 cycles of 45 sec at 94°C, 30 sec at 36°C, 1 min at 72°C, and a final extension step of 5 min at 72°C and 4ºC for the end hold. PCR products were resolved by electrophoresis in 1.5% agarose gels along with DNA Marker ladder (100-1000bp), the run was carried out at 75 Volts for approximately 60 minutes. Gels were stained with ethidium bromide, visualized under UV-light, and photographed GelDoc system (BioRad, USA). To ensure reproducibility of banding patterns, each amplification reaction was repeated twice. All the PCR reaction components and the Primers set were purchased from 3B Blackbio Biotech Biotools. The primers used for amplification of the genomic DNA are detailed in Table 1. Along with the 25 different reaction mixtures for 25 different primers, a negative control (NC - No DNA template) and a positive control (PC - No primer) were placed in thermal cycler to rule out the possibility of contamination. Reaction mixture wherein template DNA replaced by distilled water was used as negative control.

Table 1: Plant RAPD primers used for the study

No.	Primer code	Accession Numbers
1	RPl 1	AM765819
2	RPl 2	AM750044
3	RPl 3	AM773310
4	RPl 4	AM773769
5	RPl 5	AM773770
6	RPl 6	AM773771
7	RPl 7	AM773312
8	RPl 8	AM773773
9	RPl 9	AM773315
10	RPl 10	AM750045
11	RPl 11	AM911709
12	RPl 12	AM773316
13	RPl 13	AM750046
14	RPl 14	AM773774
15	RPl 15	AM773775
16	RPl 16	AM773776
17	RPl 17	AM911710
18	RPl 18	AM765830
19	RPl 19	AM773777
20	RPl 20	AM773317
21	RPl 21	AM765820
22	RPl 22	AM911711
23	RPl 23	AM911712
24	RPl 24	AM765821
25	RPl 25	AM750054

Statistical analysis:

Gelquest® and Clustervis® software were used to construct dendro-grams using the Unweighted Pair Group Method with Arithmetical Averages (UPGMA) by comparing the bands that were observed common in both plants to specific primers.

III. Results

The DNA obtained was 21.32 ng/µl (*M. pruriens*) and 20.5ng/µl (*M. cochinchinensis*).

As evident from RAPD profiles, the two accessions selected of *Mucuna* could be distinguished at the molecular level. The comparative analysis of all accessions was established on the basis of RAPD profiles generated by using decanucleotide primers. Figure 1 shows the characteristic RAPD profile of *M. pruriens*, whereas Figure 2 clearly depicts the genetic differentiation of *M. cochinchinensis*. Only the primers which displayed prominent, reproducible and distinguishable bands were considered for analysis. The 14 tested primers yielded totally 107 scorable bands with an average of three bands per primer which showed polymorphism. 60% polymorphism was observed in *M. cochinchinensis* and 92% in M. pruriens. All primers showed amplification for *M. pruriens* whereas primer number 4, 18 and 24 did not show any bands in the RAPD profile of *M. cochinchinensis.*

Figure 1: RAPD ***Mucuna* pruriens (L.) DC.**

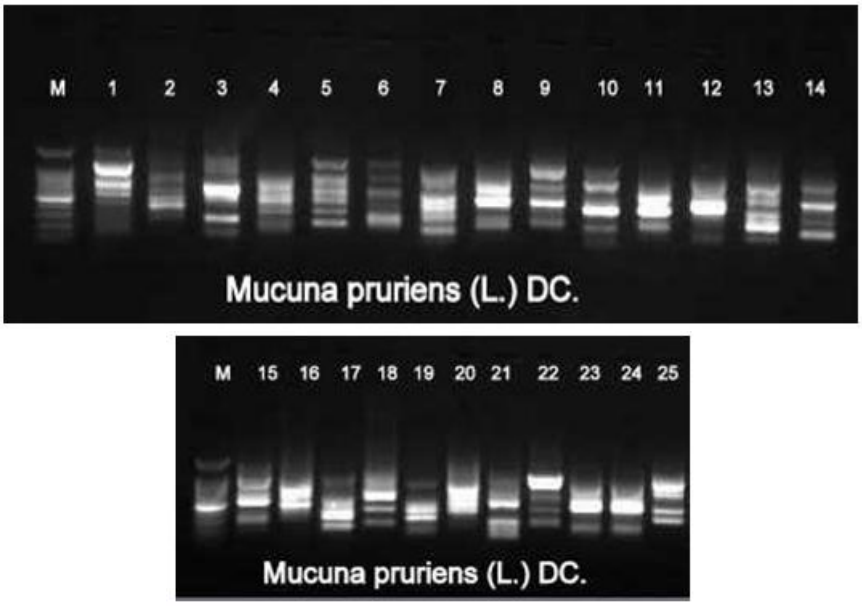
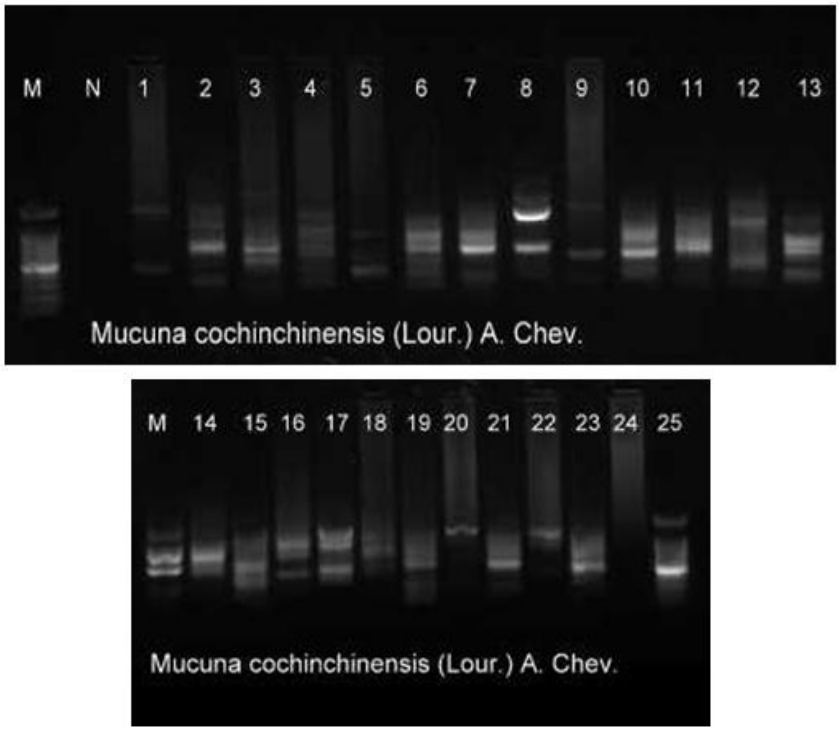
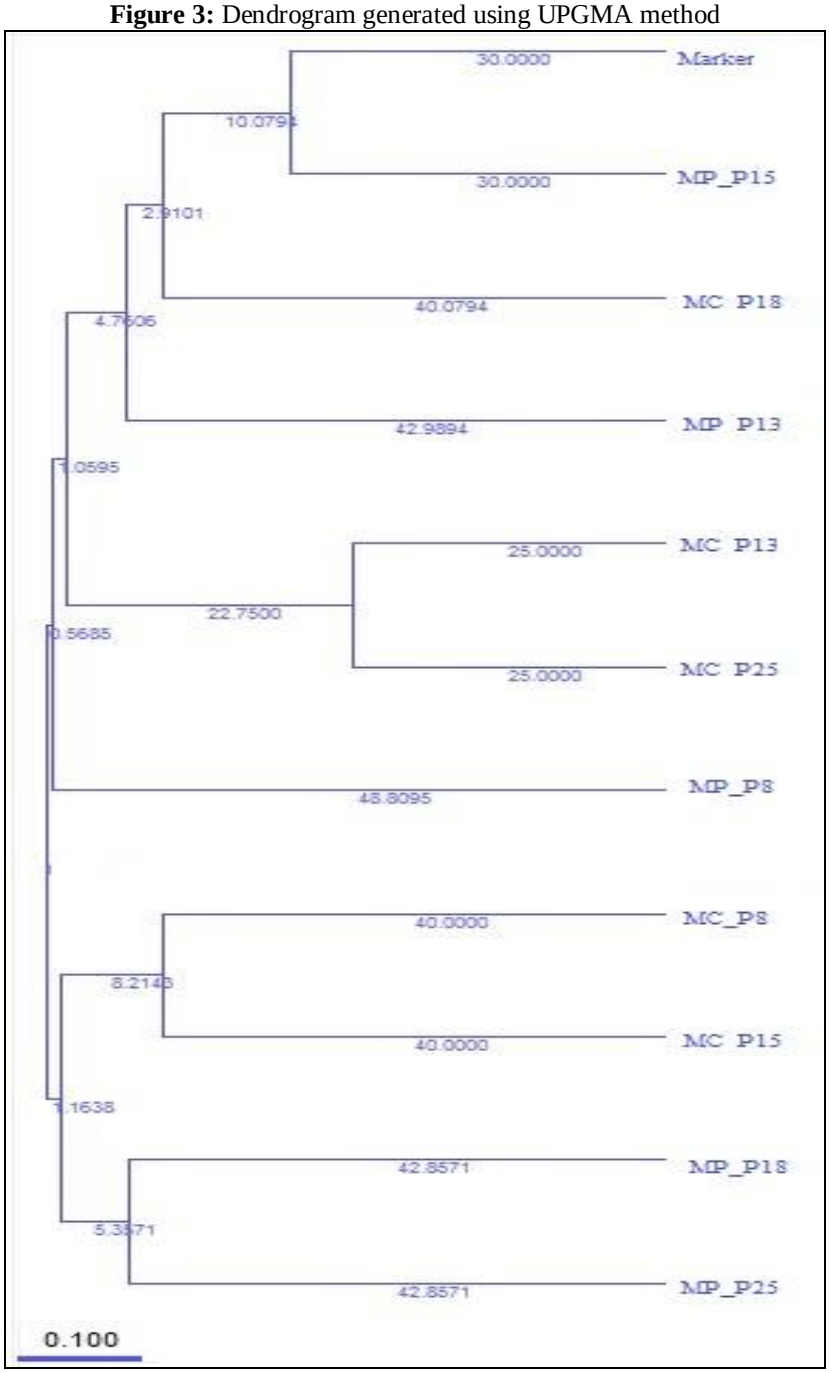


Figure 2: *Mucuna* cochinchinensis (Lour.) A. Chev.



The data scored on RAPD polymorphism and band sharing was aimed at analyzing the genetic similarity matrix obtained through multivariate analysis using Nei and Li's coefficient. The similarity coefficients were used to generate a tree for cluster analysis using the UPGMA method as evident from the phenogram (Figure 3).

Figure 3: Dendrogram generated using UPGMA method



IV. Discussion

RAPD analysis in the present study reveled very high level of Polymorphism (92%) for different *Mucuna* species. Padmesh *et al*., (2006) observed that 11 *Mucuna* accessions produced 90.1% polymorphism. Leelambika *et al*., (2010), observed 98% polymorphism in 18 accessions of *Mucuna*. Generated data showed RAPD markers are suitable tools to scan anonymous DNA of species with high secondary metabolite content such as *Mucuna*. The limited reliability of RAPD molecular markers was compensated by increasing the number of RAPD fragments. In this study, the large similarity values revealed by RAPD markers while making the phylogenetic tree provide greater confidence for assessment of genetic relationship among the species. RAPD profiling of these two plants have not yet reported.

Hadrys *et al*., (1992) used RAPD-PCR technique for studying genotype fusion at various levels, including those of individuals (parentage tests) and populations (identification of hybrid populations, species and subspecies). This profiling method was used for isolating soma-clone of altered genotypes in *Mentha arvensis* by Khanuja *et al*., 1998. The UPGMA tree showed that the two plants were related and have the common ancestral background. Though these two have different uses in Ayurvedic sciences they still can be tracked back. Based on the study the large range of similarity and dissimilarity values for the plants provides the greater confidence for assessment of genetic diversity and relationships.

V. Conclusion

The information on genetic diversity of *Mucuna* species evaluated using RAPD technique and the results provided a short and snappy view on the total genetic variability for *Mucuna* species. RAPD fingerprinting analysis is widely used in analyzing population and genetic relationships between and among species, establishing taxonomic status and evolutionary studies. Thus, this approach will be helpful in ranking the species according to their genetic inter-relatedness. This can provide a better platform for identification and authentication of *Mucuna* species. Therefore this study may contribute to provide germplasm conservationists with data in order to facilitate conservation efforts. In summary, the analysis of amplification spectra of 107 bands obtained by 25 primers clearly demonstrated the differentiation of selected accessions and established the effectiveness of this molecular tool for establishing the distinct identity of selected accessions of *Mucuna*.

Acknowledgments

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References

[1]. Albert, M.J., Bhuiyan, N.A., Talukder, K.A., Faruque, A.S.G., Nahar, S., Faruque, S.M., Ansaruzzaman, M. and Rahman, M. (1997). Phenotypic and genotypic changes in Vibrio cholerae 0139 Bengal. J. Clini. Microbio, **35**: 2588–2592.
[2]. Anonymous. (2005). The Wealth of India Raw Materials, NISCAIR Publishing New Delhi, L-M; **VI**: 439-444.
[3]. Anonymous. (2006). The Wealth of India- Raw Materials. First Suppl Ser. NISCAIR; 166-167.
[4]. Bhat, K.V., Babrekar, P.P. and Lakhanpaul, S. (1999). Study of genetic diversity in Indian and exotic sesame (*Sesamum indicum* L.) germplasm using random amplified polymorphic DNA (RAPD) markers. Euphytica, **110**: 21-34.
[5]. British Pharmacopoeia. (1973), London.
[6]. Daxenbichler, M.E., Van Etten, C.H., Hallman, E.A., Earle, F.R. and Barclay, A.S. (1971). Seeds as sources of L-DOPA. J. Med. Chem. **14**: 463.
[7]. Ding, Y., Kinjo, J., Yang, C. and Nohara, T. (1991). Triterpenes from *Mucuna* birdwoddiana. Phytochemistry **30(11)**: 3703-3707.
[8]. Eilittä, M., Bressani, R., Carew, L.B., Carsky, R.J., Flores, M., Gilbert, R., Huyck, L., St-Laurent, L. and Szabo, N.J. (2002). *Mucuna* as a food and feed crop: an overview. In: *Mucuna* as a Food and Feed: Current Uses and the Way Forward. Ed. By Flores M, Eilittä M, Myhrman R, Carew L, Carsky R, Workshop held April 26-29, 2000 in Tegucigalpa, Honduras. CIDICCO Honduras. pp. 18-46.
[9]. Hadrys, H., Balick, M. and Schierwater, B. (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol. Ecol. **1**: 55-63.
[10]. Janardhanan K. and Lakshmanan K.K. (1985). Studies on the pulse, *Mucuna* utilis. Chemical composition and anti-nutritional factors. J. Food Sci. Tech. 22: 369-37.
[11]. Khanuja, S.P.S., Shasany, A.K., Dhawan, S. and Kumar, S. (1998). Rapid procedure for isolating somaclones of altered genotypes in *Mentha arvensis*. J. Med. Arom. Plant Sci. **20**: 359-361.
[12]. Leelambika, M., Mahesh, S., Jaheer, M. and Sathyanarayana, N. (2010). Comparative evaluation of genetic diversity among Indian *Mucuna* species using morphometric, biochemical and molecular approaches. World Journal of Agricultural Sciences, **6(5):** 568-578.
[13]. Longhi, J.G., Perez, E., Jose de Lima, J. and Candido L.M.B. (2011). In vitro evaluation of *Mucuna pruriens* (L.) DC. antioxidant activity. Brazilian Journal of Pharmaceutical Sciences, **47(3)**: 535-544.
[14]. Manyam, B.V. (1995). An alternative medicine treatment for Parkinson's disease: results of a multicenter clinical trial – HP-200 in Parkinson's disease study group. J. Alternat. Comp. Med. **1**: 249-255.
[15]. Mohan, V.R. and Janardhanan, K. (1993). Studies on the Indian tribal pulses. In proceedings of the national seminar on biodiversity: strategies for conservation and future challenges. Department of Botany. Bharathiar University. Ed. by Udaiyan K, Janardhanan K, Manian S, Reddy VRKP. pp. 116-125.
[16]. Padmesh, P., Reji, J.V., Dhar, M. and Seeni, S. (2006). Estimation of genetic diversity in varieties of *Mucuna pruriens* using RAPD. Biologia plantarum, **50:**367-372.
[17]. Saini, M., Singh, S., Hussain, Z. and Yadav, A. (2010). RAPD analysis in mungbean [*Vigna radiata* (L.) Wilczek]: I. Assessment of genetic diversity. Indian J. Biotech, **9**: 137 –146.
[18]. Sastry, C.S.T. and Kavathekar, Y.Y. (1990). Plants for reclamation of wastelands. Publications and Information Directorate, New Delhi. p.317-318.
[19]. Singh, U., Wadhwani, A.M. and Johri, B.M. (1996). Dictionary of economic plants in India. Indian Council of Agricultural Research, New Delhi. p. 45-46.
[20]. Standley, P.C. and Steyermark, J.A. (1946). Flora of Guatemala. Fieldian: Botany Vol **24** Part **V.**
[21]. Wilmot Dear C.M. (1987). A revision of *Mucuna* (Leguminosae - Phaseolae) in the Indian Subcontinent and Burma. Kew Bull., **42(1)**: 23–46.

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Research article

COMPARATIVE PHARMACOGNOSTICAL EVALUATION OF SEEDS OF *MUCUNA COCHINCHINENSIS* (LOUR.) A. CHEV. (BLACK AND WHITE VARIETIES) WITH *M. PRURIENS* (L.) DC.

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ABSTRACT

M. Pruriens (L.) DC., *Mucuna cochinchinensis* (Lour.) A. Chev. var. black and white are reported to contain chemical compound L-dopa, which is used in the treatment of Parkinson's disease. Aqueous extract of seeds of these species showed aphrodisiac properties. Many Ayurvedic practitioners are using the seed powder to treat disease like gonorrhoea, sterility and general debility. Seeds of these species were collected, shade dried and kept in airtight container and preserved in FAA for microscopic evaluation. Shade dried seeds were made in to powder and sieved through #60. Characters such as color, size, shape, weight as well as microscopic characters observed through transverse section and powder microscopy have also been described in detailed. Result revealed that microscopic characters such as, thickening of lumen of palisade cells of testa, number of layers of spongy parenchyma of testa region and number of mesophyll layers of cotyledon were the main distinguishing characters of selected *Mucuna* species.

KEY WORDS: *Mucuna* species, pharmacognosy, macroscopy, microscopy, powder microscopy, Ayurveda

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INTRODUCTION:

Indian system of Medicine, particularly Ayurveda, prescribes 80% of their medicines, which are plant origin (Anonymous, 2003). The demand of Ayurvedic or herbal medicine is increasing day by day in the developed countries due to its safety and efficacy. Now a days, there are, plenty of formulations and preparations available in the markets in the name of vitalisers and aphrodisiac products. Increasing demand for aphrodisiac products in the global market, lead the industry to prepare neutraceuticals which does not require approval of the drug controllers (Ramawat and Goyal, 2008).

There are 105 species of *Mucuna* available in the tropical parts of the Earth and about 15 species found in India (Anonymous, 1962). Plants of this genus belong to family fabaceae and are annual or perrenial twinner herb or shrub (Mebberly, 2008). Among these species, *Mucuna pruriens* (L.) DC. has been reported as an aphrodisiac drug and known as *Kapikacchu* in Ayurveda (Sharma *et al*., 2000). *Mucuna pruriens* is an ingredient of Ayurvedic formulations *Shakuladi sidhmala, Pana, Bhakshana* which is used in the treatment of rheumatism (Parmar *et al.,* 2016). Pharmacognostic studies on seed and root of *Mucuna pruriens* have been reported by Yelne and Sharma, (1992); Chauhan and Pillai (2011). According to Garg (1992), other species namely, *Mucuna utilis* Wall ex Wight are being sold in the name of *Kapikacchu*.

A literature survey revealed that, there are other species of *Mucuna viz*., *Mucuna cochinchinensis* (Lour.) A. Chev. white seeded from Northen parts and *Mucuna cochinchinensis* (Lour.) A. Chev. black seeded varieties from Southern parts of India are also used by Ayurvedic physicians and sold in the market in the name of *Kapikacchu*. Murthy *et al*. (2015), reported that the *M. cochinchinensis* black seed variety contains maximum L-dopa as compared to the other species of *Mucuna*.

Keeping in view, the variation in seed, leaf, pod and plant morphology; a comparative account of seed pharmacognosy has been performed. This communication deals with the macroscopic, microscopic and powder microscopic evaluation of *M. pruriens*, *M. cochinchinensis* black and white seed varieties.

MATERIAL AND METHODS:

Plant material:

Seed material of *Mucuna pruriens* (L.) DC, *Mucuna cochinchinensis* (Lour.) A. Chev. (White seed) procured from Pune market and *Mucuna cochinchinensis* (Lour.) A. Chev. (Black seed) from Kerala market were authenticated from Agharkar Research Institute, Pune, Maharashtra, India, with voucher number S-162, S-161 and S-159, respectively. Above seeds were sown at Institute's garden and thereafter saplings were further cultivated. The fresh materials have been used from these cultivated sources for further pharmacognostical studies.

Herbarium preparation:

Herbarium specimens were prepared and preserved in the Herbarium section of the Institute with voucher specimen number *Mucuna cochinchinensis* (Lour.) A. Chev. (White seed)- 4476, *Mucuna cochinchinensis* (Lour.) A. Chev. (black seed) – 4487 and *Mucuna pruriens* (L.) DC.- 4475.

Preparation of wet sample:

Fresh seeds were collected from the plants cultivated in institute garden. Seeds were thoroughly washed and kept separately in a glass bottle containing a mixture of Formalin: Glacial Acetic Acid: 70% Ethyl alcohol [10:5:85] (Johansen, 1940).

Powder preparation:

Shade dried seeds of *Mucuna* species were made into powder with the help of grinding mill. Powder was sieved through #60 mesh and kept in a air tight container for further analysis.

Morphological characters of seed:

Morphological or organoleptic characters like shape, size, taste, odour, colour of the three pods, seeds and powder were recorded as seen from naked eyes (Kokate *et al.*, 2008).

Microscopic characters of seeds:

Free hand sections (T.S.) of each material were taken and stained with Phloroglucinol plus Hydrochloric acid and Iodine. Micro photographs were snapped with the help of DenoCapture 2.0 version 1.4.2.D the versatile digital microscope. The histological studies were carried out as per the methods described by Johansen, 1940.

Powder microscopy of seed:

Pinch of powder taken in watch glass and stained with Phloroglucinol plus Hydrochloric acid and Iodine. Micro-slides were prepared and observed under trinocular, Biolux make. Micro photographs were snapped with Deno Capture 2.0 version 1.4.2.D the versetile digital microscope.

RESULTS AND DISCUSSION:

Morphological characters of seed:

Seeds of *Mucuna* were ovate to oblong with hard, glossy surface; hilum conspicuous more or less elongated with distinct slit, cushioned with white spongy tissue and bordered with aril, located at one end of the longer edge of the seed near micropylar end. Details of comparative account of these species have been depicted in the Table-1, 2 and Figure-1

Table-1: Comparative morphological characters of plants of *Mucuna* species

Morphometric characters	*M. cochinchinensis* (Lour.) A. Chev. var. Black	*M. cochinchinensis* (Lour.) A. Chev. var. White	*M. pruriens* (L.) DC.
Habit	Annual twiner	Annual twiner	Annual twiner
Flower	white or pale purple	Dark purple	purple
Pod	10–15 cm long, 1–2.5 cm wide, green with black bristles when young, black, wrinkled and destitude of down when ripe, bristles not irritant	10–15 cm long, 1–2.5 cm wide, green with white bristles when young, black, wrinkled and destitude of down when ripe, bristles not irritant	5–10 cm long, 1.5–1.8 cm wide, light green with white bristles when young, pale brown or grey, longitudinally ribbed, turgid, bristles irritant.
Seed	Brown to black, 6–8 in a pod.	Dull white, 6–8 in a pod.	Black, 4–6 in a pod.

FIGURE 1: Comparative morphological characters of *Mucuna* species

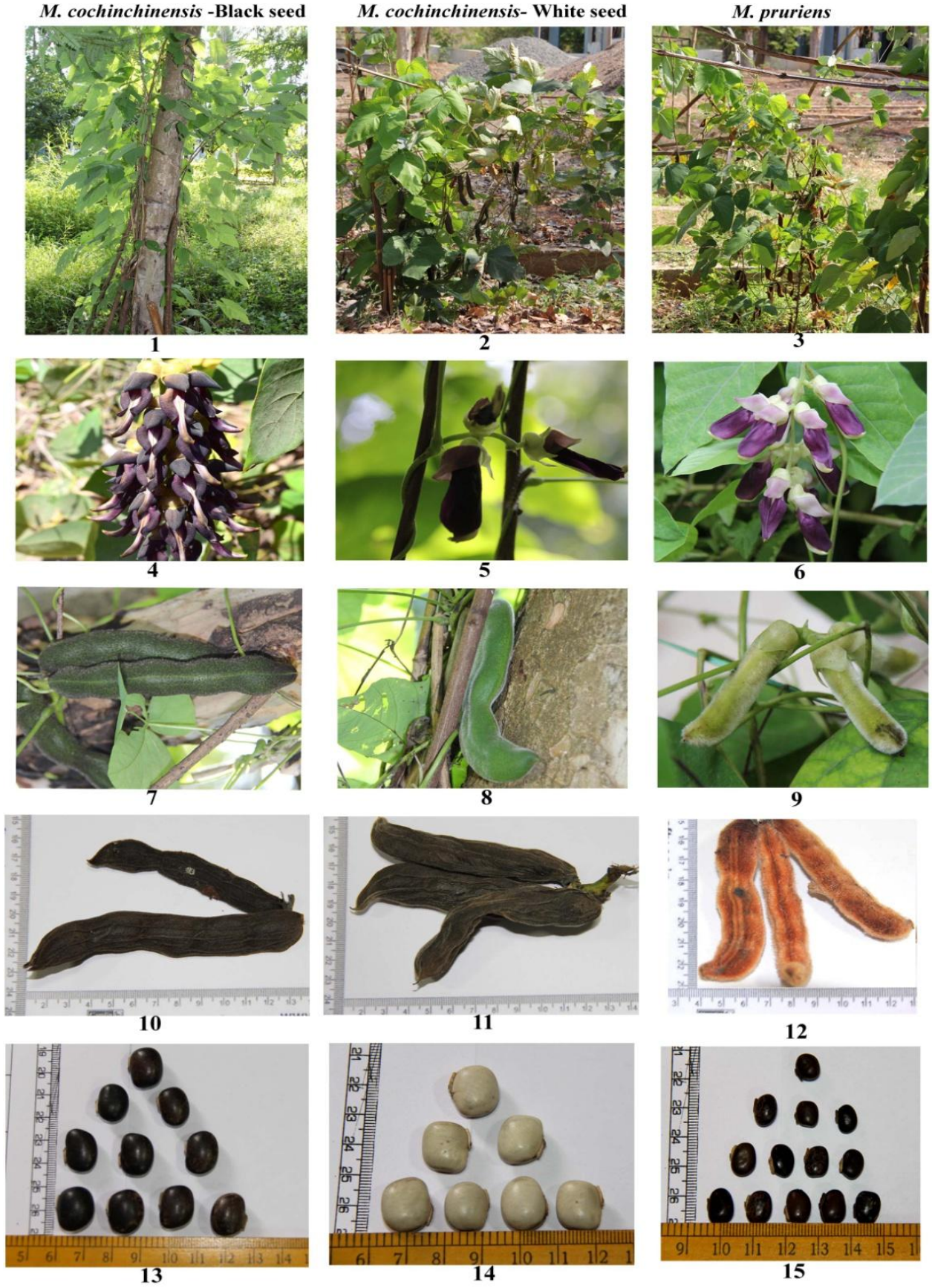


Figure 1: 1,2,3- Habit; 4,5,6- Flowers; 7,8,9- Fresh pods; 10,11,12- Mature dried pods; 13,14,15- Mature dried seeds

Table-2: Comparative morphological characters of seeds of *Mucuna* species

Macroscopic characters	*M. cochinchinensis* (Lour.) A. Chev. var. Black	*M. cochinchinensis* (Lour.) A. Chev. var. White	*M. pruriens* (L.) DC.
Shape	ovate, oblong, globose	ovate, oblong, globose	ovate, oblong, flattened
Size	length -1.25–2.19 cm, breadth- 0.93–1.5 cm, thickness -0.45–1.16 cm.	length -1.20–2.0 cm breadth- 0.95–1.37 cm thickness -0.50–1.0 cm	length -0.8–1.29 cm, breadth- 0.6–0.9 cm, thickness -0.3–0.63 cm
Surface	hard, smooth, glossy	hard, smooth, glossy	hard, smooth, glossy
Colour	brown to black	dull white	brown to black
Hilum	conspicuous, 0.6–0.8 cm long	conspicuous, 0.7–0.9 cm long.	conspicuous 0.4–0.5 cm long.
Weight of seed	Upto 2.7 gm	Upto 1.9 gm	Upto 0.5 gm
Powder characters			
Colour	creamish gray	yellowish cream	creamish gray
Texture	course	course	course
Odour	odourless	odourless	odourless
Taste	starchy - slightly bitter	starchy -slightly bitter	starchy - slightly bitter

Microscopy:

Detailed T. S. of seed passing through testa:

Transversed section (TS) of *Mucuna species* was oval in shape. TS showed outer most single, malpighian layer of testa, composed of thick walled cylindrical palisade cells covered with thin cuticle. A line of linea lucida could easily be seen across these cells; followed by a layer of spool shaped cells having apical and basal ends; both ends connected with long, vertically running narrow strip. Underneath this lies the spongy parenchyma layer. Major differences among the microscopic characters of testa of seeds have been exhibited in Table-3 and Figure-2.

Table-3: Comparative account of microscopic characters of *Mucuna* species

Microscopic characters	*M. cochinchinensis* (Lour.) A. Chev. var. Black	*M. cochinchinensis* (Lour.) A. Chev. var. White	*M. pruriens* (L.) DC.
Palisade cells of testa	Lumen narrow and broadest at apical end	Lumen narrow and broadest at apical end and basal end.	Lumen narrow and broadest at basal end.
Spongy parenchyma of testa	No. of layers 18–20 outer 7–8 layers small with few inter cellular spaces Middle 8–9 layers elongated cells with compact arrangement inner 3–4 collapsed layers	No. of layers 14–16 outer 7–8 layers big with inter cellular spaces Middle 5–6 layers with few inter cellular spaces inner 3–4 collapsed layers	No. of layers 9–16 outer 7–8 layers small, compact middle 5–6 layers elongated, compact inner 3–4 collapsed layers
No. of mesophill layers in cotyledon	up to 30	up to 35	up to 20

FIGURE 2: Comparative account of detailed T. S. of seed passing through testa

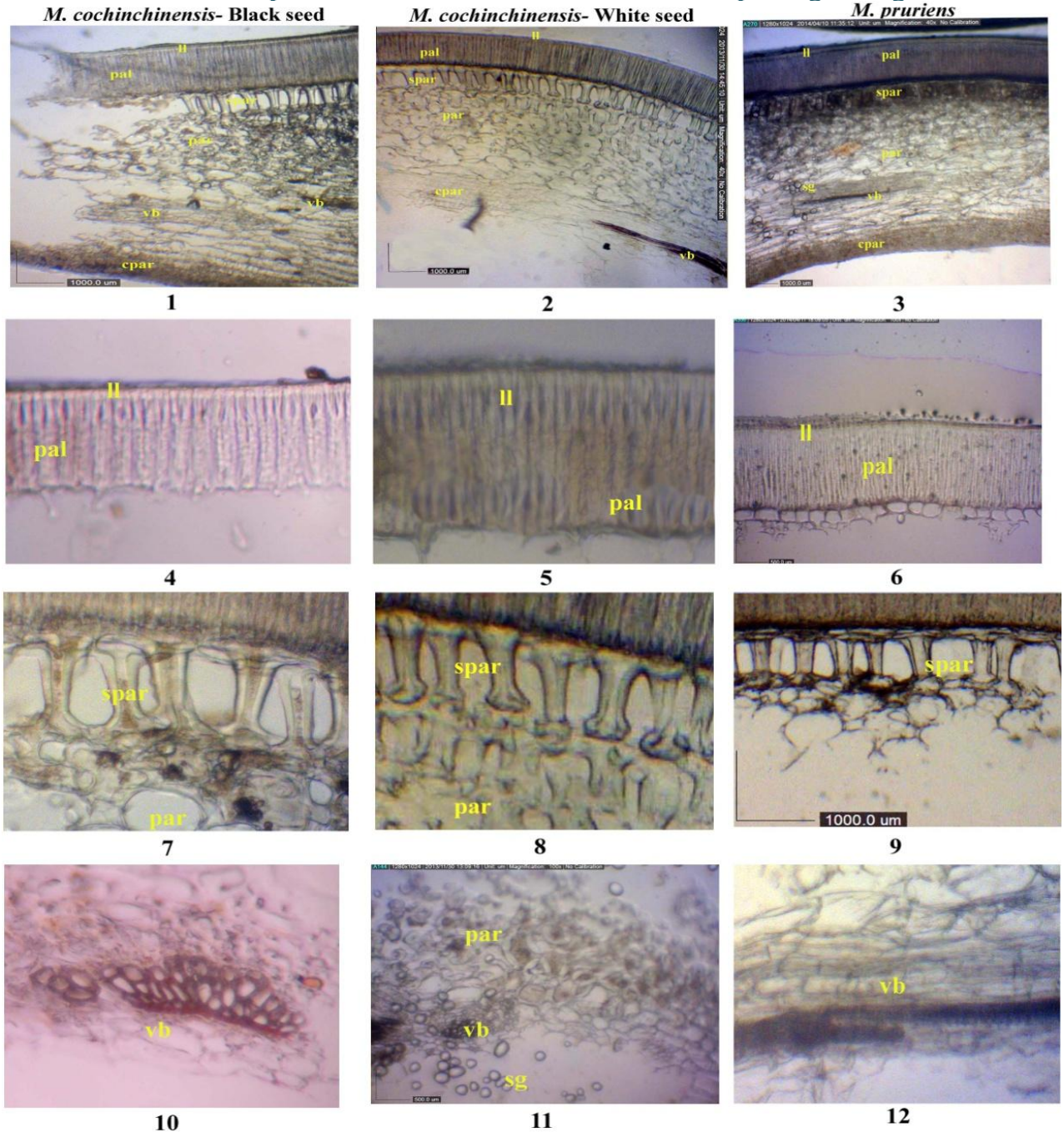


Figure 2: 1,2,3- Transverse section of seed passing through testa; 4,5,6- Enlarge view of palisade showing linea lucida; 7,8,9- Enlarge view of spool shaped parenchyma; 10,11,12- Enlarge view of testa showing parenchyma traverse with vascular bundle.

Detailed T. S. of seed passing through cotyledon:

Transverse section exhibed outer and inner epidermis, made up of squarish to rectangular cells, embeded with aleurone grains. Cells of inner epidermis observed to be bigger in size as compared to the cells of outer epidermis. In between, outer and inner epidermis, cells of mesophyll present, containig starch grains and aleurone grains and traversed with developed and undeveloped vascular bundles. Detailed comparative account among these species is given in Table –3 and Figure- 3.

FIGURE 3: Comparative account of detailed T. S. of seed passing through cotyledon

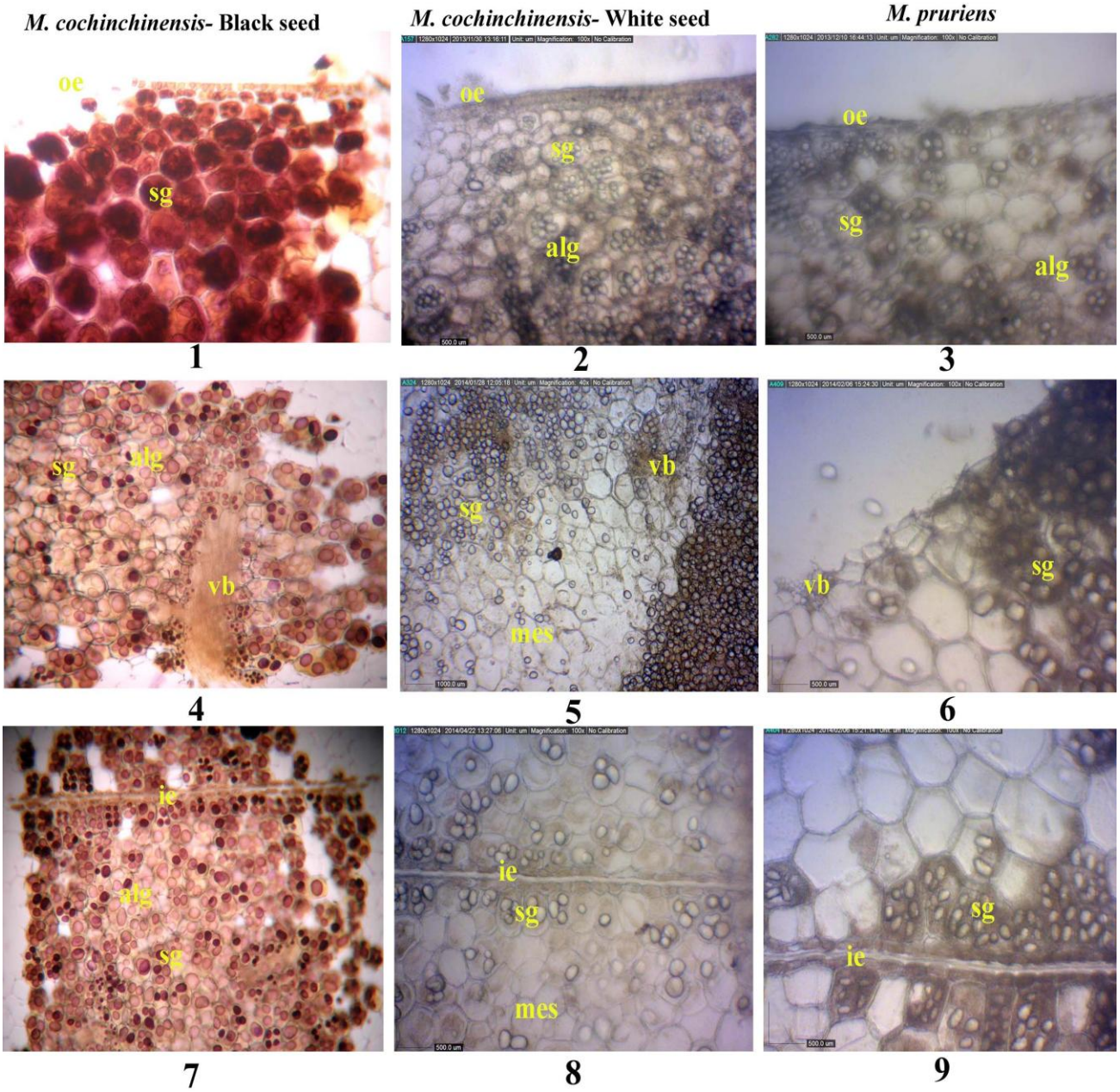


Figure 3: 1,2,3- TS of cotyledon showing outer epidermis; 4,5,6- TS of cotyledon showing middle mesophyll cells; 7,8,9- TS of cotyledon showing inner epidermis.

Detailed T. S. of seed passing through hilum region:

Section showed two layered palisade cells containing brown content. Below this, irregular parenchyama layers, traversed with developed and undeveloped vascular bundles and few layers collapsed at the lower end. Tracheidal bar found embedded in it at narrow groove run from the centre of two rows of palisade cells. Arillus was lying adjacent to the palisade cells. Details are shown in Figure -4.

FIGURE 4: Comparative account of detailed T. S. of seed passing through hilum region

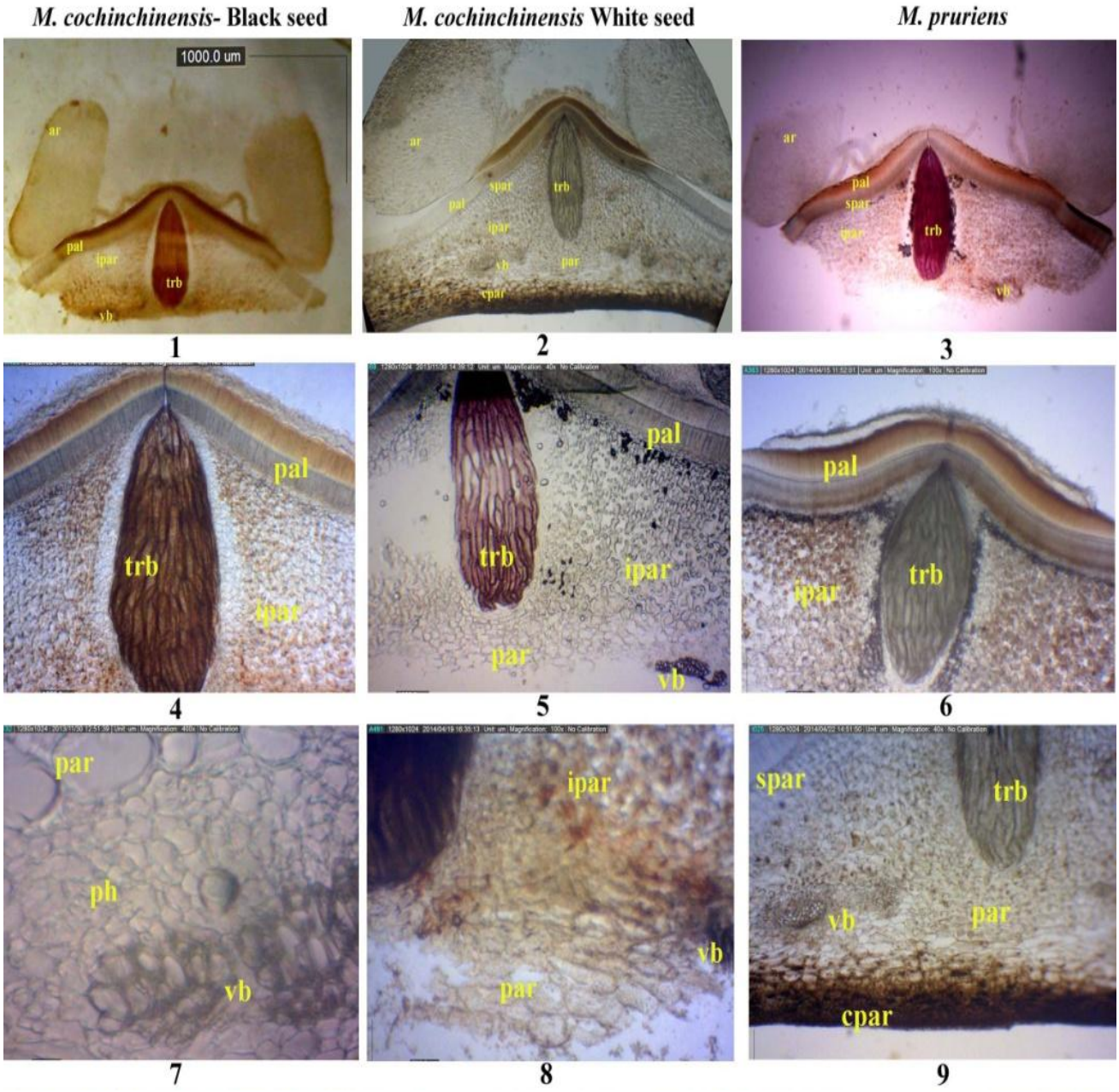


Figure 4:1,2,3-Transverse section of seed passing through hilum; 4,5,6- Enlarge view showing double layered palisade, tracheidial bar and irregular parenchyma; 7,8,9- Enlarge view showing lower parenchyma and vascular bundle.

Powder microscopy of seeds-

Powder showed palisade cells in surface view seen from above and below, 2 layers of palisade cells from the hilum region, spool shaped cells in surface view, spiral vessels, isolated cells of arillus, isolated starch grains from cotyledons, fragment of tracheidal bar, isolated starch grains from radical (Figure 5 and 6).

FIGURE 5: Comparative cellular details observed in powder microscopy of seed

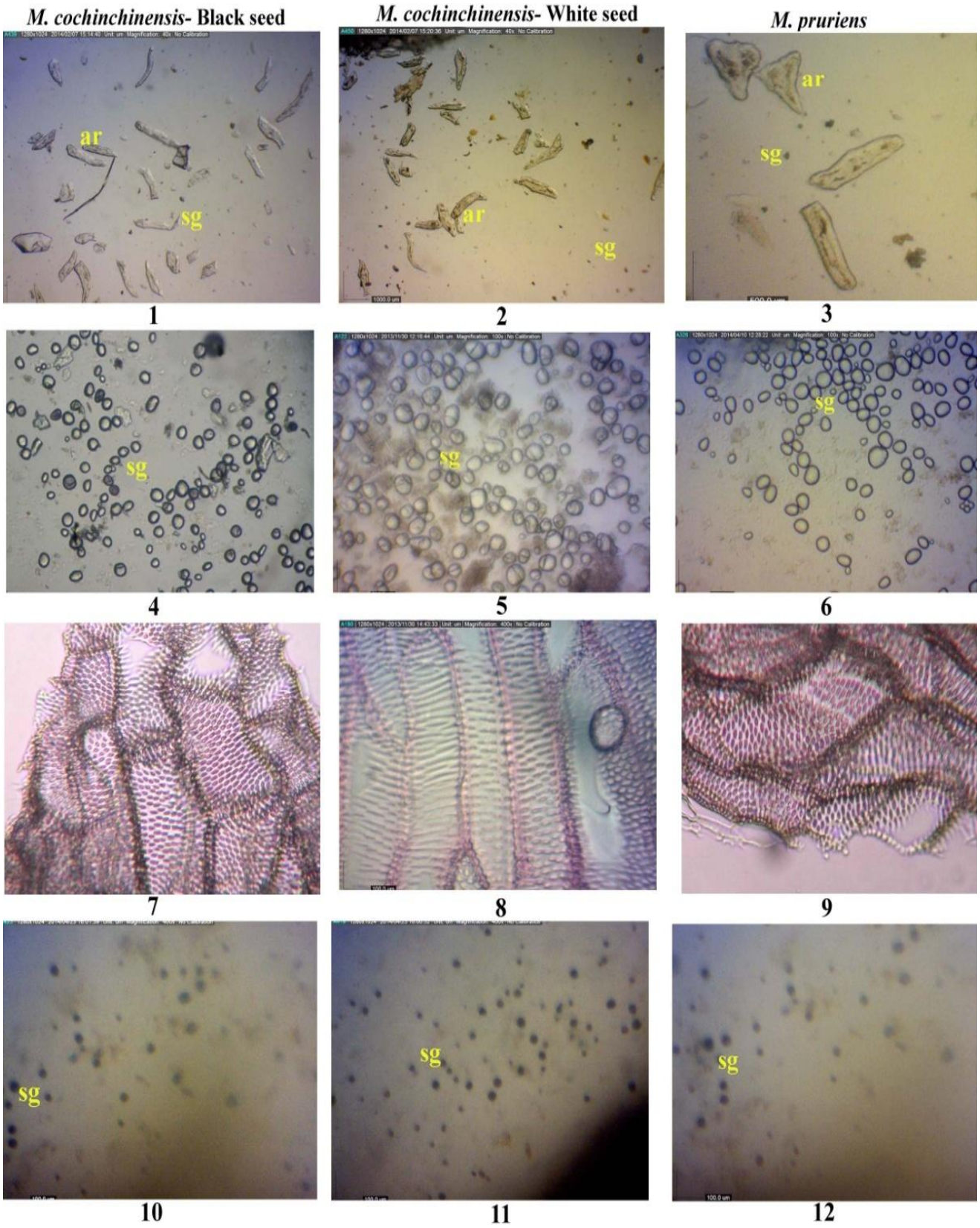


Figure 5: 1,2,3- Isolated cells of arrilus; 4,5,6- Isolated starch grains from cotyledon; 7,8,9- Fragment of tracheidal bar; 10,11,12- Isolated starch grains from radical.

FIGURE 6: Comparative cellular details observed in powder microscopy of seed

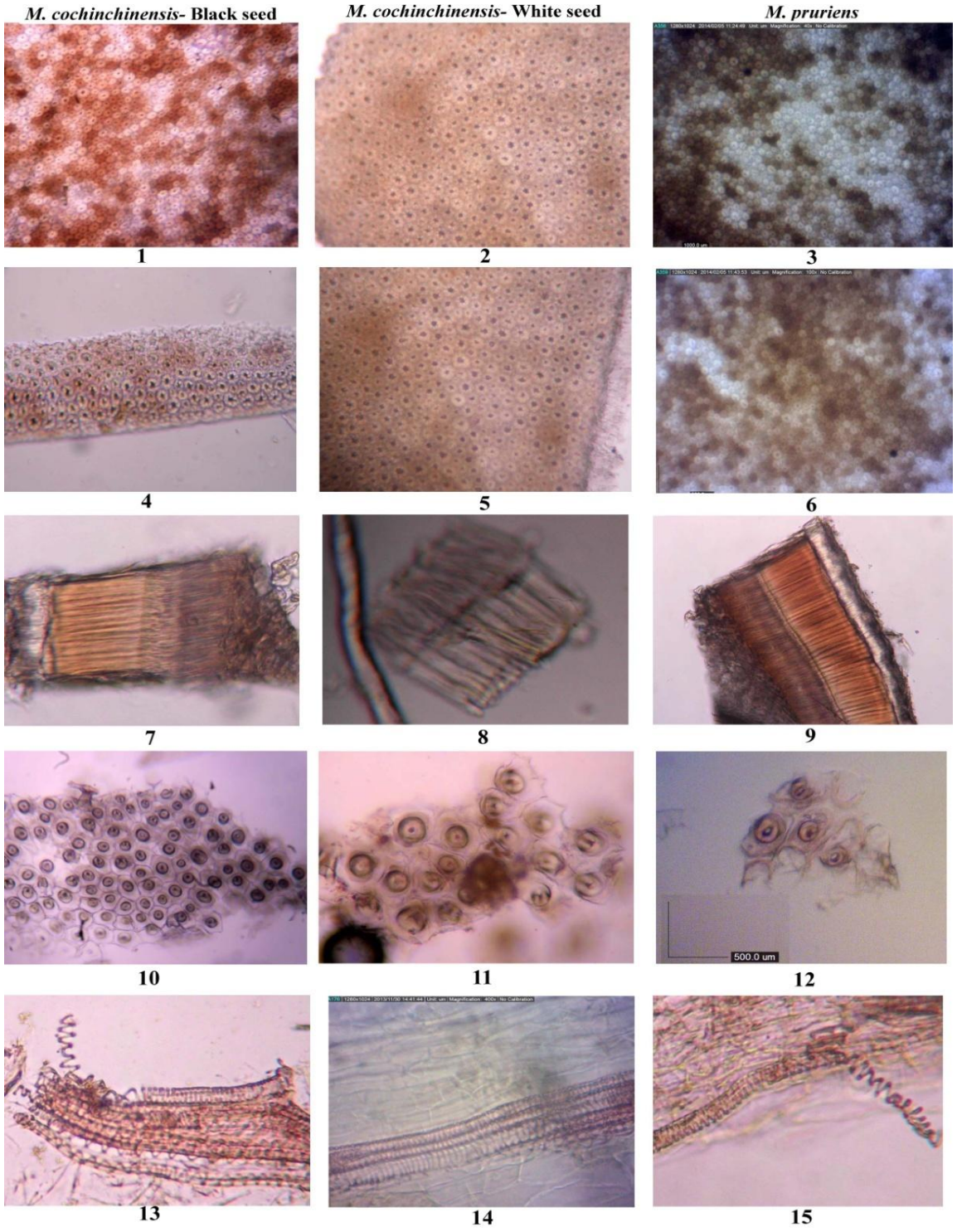


Figure 6: 1,2,3- Palisade cells in surface view seen from above; 4,5,6- Palisade cells in surface view seen from below; 7,8,9- 2 layers of palisade cells from the hilum region; 10,11,12- Spool shaped cells in surface view; 13,14,15- Spiral vessels.

Abbreviations- alg- aleurone grains, ar- arillus, cpar- collapsed parenchyma, ct- cortex, e- epidermis, ie- inner epidermis, ipar- irregular parenchyma, ll- linea lucida, oe- outer epidermis, pal- palisade, par- parenchyma, sg- starch grains, spar- spool shaped parenchyma, trb- tracheidal bar, uvb- undeveloped vascular bundle, vb- vascular bundle.

DISCUSSION

Yelne and Sharma (1992) and Chauhan and Pillai (2011) reported presence of palisade layer columnar or spool shaped cells, tracheids and reticulate, spiral vessels in the seed powder of *M. pruriens* (L.) DC. Our findings were also at par with them; except that Yelne and Sharma (1992) had not reported the presence of double layered palisade cells, palisade cells in surface view seen from above and below; columnar cells in surface view. Whereas isolated cells of arillus and isolated starch grains from radical region of seed were not reported by both the authors, but newly found and being reported in the present communication.

We found major differences in morphological characters of seed of *M. cochinchinensis* (Lour.) A. Chev. var. Black, *M. cochinchinensis* (Lour.) A. Chev. var. White and *M. pruriens* (L.) DC., in respect of size, shape, color and weight as well as in microscopic characters such as, thickening of lumen of palisade cells of testa, number of layers of spongy parenchyma of testa region and number of mesophyll layers of cotyledon.

CONCLUSION

The resultant data obtained through experimental exercise such as, pharmacongnostic evaluation of *M. cochinchinensis* (Lour.) A. Chev. var. Black, *M. cochinchinensis* (Lour.) A. Chev. var. White and *M. pruriens* (L.) DC. will be useful for the identification and authentication of correct *Mucuna* species. The differences in morphological and microscopical characters among these species would also be helpful to detect the possible adulteration in the genuine drugs of *Kapikacchu* (*Mucuna* species).

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REFERENCES

Anonymous (1962). The Wealth of India. Raw materials L-M. New Delhi: CSIR. p. 439.

Anonymous (2003). Export Potential of Indian Medicinal Plants and Products. Mumbai: Quest publications. p. 10.

Chauhan M, Pillai APG (2011). Microscopic profile of drugs used in Indian systems of medicines. vol.-3 Seed drugs part 1. Jamnagar: PGT SFC cell IPGT&RA, GAU. p. 107–108.

Garg S (1992). Substitute and adulterant plants. New Delhi: Periodiacal experts Book Agency.p. 82

Johansen DA (1940). Plant Micro Techniques. New York London: McGrow Hill Book Company. p.105.

Kokate CK, Purohit AP, Gokhale SB (2008). Pharmacognosy. 42nd ed. Pune: Nirali Prakashan. p. 6.3.

Mebberly DJ (2008). Mebberly's Plant Book-A portable dictionary of plant, their classification and uses. 3rd edition. New York: Cambridge University Press. p. 561.

Murthy SN, Malgaonkar MM, Shirolkar AR, Pawar SD, Sangvikar S, Kulkarni YR (2015). A comparative assessment of pharmacologically active principles and antioxidant activity of commonly occuring *Mucuna* sps. in India. International Journal of Ayurveda and Pharma Research. 3(11): 8–13.

Parmar N, Singh S, Patel B (2016). Various pathya kalpana of Bilva [*Aegle marmelos* (L.) Correa ex Roxb.] - A review, Global J Res. Med. Plants & Indigen. Med. 5(2): 57–78.

Ramawat KG, Goyal S (2008). The Indian Herbal Drugs Scenario in Global Perspectives, In: Bioactive Molecules and Medicinal Plants in Ramawat KG, Merillon JM (eds.) Udaipur, India: Springer.p. 323–345.

Sharma PC, Yelne MB, Dennis TS (2000). Database on Medicinal Plants used in Ayurveda,Vol.-1. New Delhi: Central Council for Research in Ayurveda and Siddha.p. 200–204.

Yelne MB and Sharma PC (1992). Pharmacognostic study of Kapikacchu (*Mucuna pruriens* (L.) DC.) Root and seed. Bulletin of Medico- Ethno-Botanical Research. 13(1–2):52–64.

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