

**ESTIMATION L-DOPA CONTENT OF *Mucuna pruriens* SEEDS BEFORE AND
AFTER PURIFICATION**

MIROSH DINUSHA PARANAGAMA JAYASUNDARA

Submitted in partial fulfillment of the requirement
for the award of the degree of

**MASTER OF SCIENCE IN INDUSTRIAL UTILIZATION OF MEDICINAL
AND AROMATIC PLANTS**

of the

UNIVERSITY OF SRI JAYAWARDENEPURA

SRI LANKA

2010

DECLARATION

I do hereby declare that the work reported in this project report/thesis was exclusively carried out by me under the supervision of Prof. Ajith Abeysekera. It describes the result of my own independent research except where due reference has been made in the text. No part of this project report/thesis has been submitted earlier or concurrently for the same or any other degree.

Date. 02. / 08. / 2010....

.....


Signature of the Candidate

Certified by:

1. Supervisor (Name):.....

Date:.....

(Signature):.....

2. Co-Supervisor (Name):.....

Date:.....

(Signature):.....

ABSTRACT

In Ayurvedic medical system "Cowhege" (*Mucuna pruriens* Linn) belonging to the family Fabaceae is used for male sexual disorders since ancient time. The plant's efficacy in treating sexual disorders has been documented in ayurveda. As mentioned in ayurvedic texts *Mucuna pruriens* seeds are mainly used as the medicinal component. The texts have also mentioned that these seeds should undergo a purification process before they are used as drugs. During the purification seeds are boiled with cow's milk and seed coats and pumule are removed. A vast number of chemical compounds can be found in these seeds. The main chemical compound which helps to improve male fertility is 3, 4-dihydroxy phenylalanine (L-DOPA)

During my research I attempted to determine L-DOPA content of the seeds before and after purification. Pure L-DOPA was isolated from Syndopa tablets using crystallization method. TLC was performed on ethanol extract of the seed samples. Ethanol extract of pure L-DOPA was prepared as a dilution series and TLC was performed. (Solvent system: n-Butanol-Acetic acid-Water 4:1:1). TLC results of seed samples were compared with TLC results of pure L-DOPA dilution series, by using Eye Estimation method.

Concentration of unpurified sample was approximately between, 42 gl^{-1} and 54 gl^{-1} . Concentration of purified sample was approximately between, 18 gl^{-1} and 30 gl^{-1} .

According to the results of this study it was revealed about 50% of L-DOPA is reduced during the purification process.

ACKNOWLEDGEMENT

It is with great pleasure that I place on record my deepest gratitude to my supervisor Prof. A. M. Abeysekera, Dean, faculty of Applied science, university of Sri Jayewardenepura, Nugegoda, for his invaluable advice and guidance provided throughout the study and for giving the needed inspiration and encouragement to successfully complete this project.

I wish to thank Dr. Champa Jayaweera, Co-supervisor, Lecturer, faculty of Applied science, university of Sri Jayewardenepura, Nugegoda, for the support given during literature review and carrying out experiments.

I also express my sincere thanks to Prof. S.I.Samarasinghe, Course coordinator, lecturer, department of Chemistry, university of Sri Jayewardenepura, Nugegoda, for giving support to carry out all the experiments and providing encouragement to successfully complete this project.

I must record my special thanks Dr. T.M.S.G. Thennakoon, Visiting lecturer, faculty of Applied science, university of Sri Jayewardenepura, Nugegoda, who taught me about the densitometer and how to operate the machinery.

I also express my sincere thanks Dr.A.M.J. Wanshapala, Lecturer, department of Food science, university of Sri Jayewardenepura, Nugegoda, for giving me support to find out literature.

I wish to express my sincere thanks Mr. R.M.S.R Jayawardena and all academic and non academic staff at department of Chemistry, university of Sri Jayewardenepura, Nugegoda, who gave support during laboratory work and for the completion of the project report.

I wish to express my heartfelt gratitude for my parents and my family who have been a source of encouragement throughout the study.

Finally, I would like to thank all others who helped me in numerous ways to make this study a success.

Table of Contents

Declaration	ii
Abstract	iii
Acknowledgment	iv
Table of contents	vi
List of Tables	viii
List of Figures	ix
List of Abbreviations	x
CHAPTER 1 – INTRODUCTION	01
1.1. <i>Mucuna pruriens</i> Linn. (Family FABACEAE)	02
1.1.1 Medicinal properties of <i>Mucuna pruriens</i> seeds	05
1.1.2 Therapeutic evaluation of seed	06
1.1.3 Pharmacognosy of seed	06
1.1.4 Chemical and physical constituent of <i>Mucuna pruriens</i> seed	06
1.2 Infertility	08
1.2.1 Ayurvedic concept of infertility	08
1.2.2 Modern concept on infertility	10
1.2.3 Male infertility	12
1.3 Shodana (Purification)	14
1.3.1 Purification of <i>Mucuna pruriens</i> seeds	16

1.4 Levodopa	17
1.5 Cow's milk	18
1.6 Previous Studies	20
CHAPTER 2 - METHODOLOGY	
2.1 Isolation of L-Dopa	23
2.2 Sample collection	23
2.3 Purification of seeds	23
2.4 Preparation of sample extracts	24
2.5 Preparation of dilution series	25
CHAPTER 3 - RESULT AND DISCUSSION	27
CHAPTER 4 - CONCLUSION	30
CHAPTER 5 - SUGGESTION FOR FURTHER STUDIES	31
REFERENCES	32
Appendix 1	
Instrument and Chemicals	35

List of Tables

Table 1.1	Composition of Cow's milk	19
Table 2.1	Dilution series of pure L-Dopa	25
Table 2.2	Sample concentration of dilution series	26

List of figures

Figure 1.1 Inflorescence of <i>Mucuna pruriens</i> plant	03
Figure 1.2 Pods of <i>Mucuna pruriens</i> plant	04
Figure 1.3 Seeds of <i>Mucuna pruriens</i>	04
Figure 1.4 Chemical structure of Levodopa	17
Figure 2.1 Boiling of <i>Mucuna pruriens</i> seeds with Cow's milk	24
Figure 2.2 Purified <i>Mucuna pruriens</i> seeds with peel	24
Figure 3.1 TLC results of the samples (under 254 nm)	27
Figure 3.2 TLC results of the dilution series (under 254 nm)	28

List of Abbreviations

TLC	Thin Layer Chromatography
L- Dopa	Levodopa
R _f	Rate of flow
HPTLC	High-performance thin layer chromatography
UV	Ultra violet
WHO	World Health Organization
Kcal	Kilo-calorie
KJ	Kilo-joule
mg/ml	milligrams per milliliter
g/l	grams per liter

Chapter 1

INTRODUCTION

Indigenous herbs are used as remedies against various diseases in the traditional system of medicine or in ethno medical practices. For the past few decades compounds from natural sources have gained importance, because of the vast chemical diversity that they offer. This has led to phenomenal increase in the demand for the herbal medicine in the last two decades and a need to ensure the quality, safety and efficacy of the herbal drugs. Phyto-chemical evaluation is one of the tools for the quality assessment, which includes preliminary phyto-chemical screening, chemo profiling and marker compound analysis using modern analytical techniques.

Mucuna pruriens is commonly known as “the cowhage” or “velvet” bean and “atmagupta” in India. It is a climbing legume endemic in India and also in other parts of tropics including Central and South America. In ayurvedic system of medicine, *Mucuna pruriens* is used for the management of male infertility, nervous disorders and also as an aphrodisiac. Different preparations of the seeds are also used for the management of ageing, rheumatoid arthritis, diabetes, male infertility and nervous disorders. *Mucuna pruriens* seed powder contains high amount (25%) of L-DOPA, which is a neurotransmitter precursor^[1]. Therefore this compound was used as a marker compound during this research. In addition to that levodopa is also the main chemical, which helps to improve male fertility.

During this research the levodopa content was determined in both unpurified and purified seeds. Since pure levodopa is not available in Sri Lanka, Syndopa, a mixture of

levodopa and carbidopa was used and levodopa was isolated by using crystallization method.

Research has not been performed on the chemical changes which occur due to the purification process. This research was performed to analyze if any chemical change take place during purification by analyzing the levodopa content in the unpurified and purified *Mucuna pruriens* seeds.

A large number of chemicals can be found in the seeds, therefore it will be very complicated and complex to check each and every chemicals change. Hence one major chemical compound, levodopa, was used as the maker compound.

1.1 *Mucuna pruriens* Linn. (Family FABACEAE)

Some synonyms, morphological description and some ayurvedic concepts of *Mucuna pruriens* are mentioned below.

Synonyms^[2]

Sinhala	-	Vanduru me
Sanskrit	-	Kapikachchu, Atmagupta, Kandura, Vyanga.
English	-	Common cowitch, Cowhage,
Tamil	-	Punaikkali, Amudari.
Hindi	-	Kaunch, Kevach, Kevanch.
Urdu	-	Kavancha

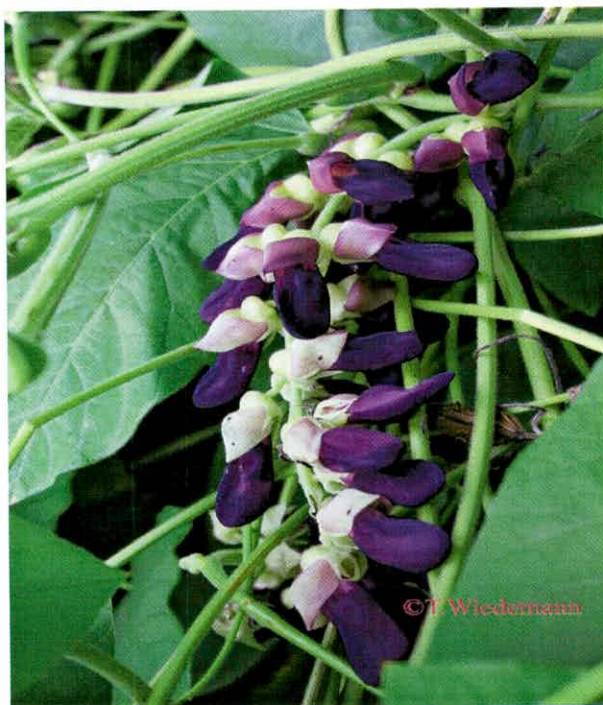


Figure 1.1 Inflorescence of the *Mucuna pruriens* plant

Mucuna pruriens grows in wild areas of the Himalayan foothills and the plains of Punjab to Sri Lanka. The plant is a slender climbing annual. The leaves are 15-22 cm long, leaflets 7-12 cm long, ovate rhomboid membranous and the petiole is 6-11 cm long. The inflorescence is racemes. The corolla is purplish. The inflorescence of *Mucuna pruriens* is shown in fig 1.1. The pod is 5-7 cm long, bristly red curved or golden colored. Used parts of *Mucuna pruriens* are roots, leaves, seeds, and bristles.^[2]

Glands are presented on bristles. The pods of *Mucuna pruriens* are showed in fig 1.2. The seeds are bean shaped, white black or spotted and about 1 cm in diameter. The picture of seeds is shown in fig 1.3.^[3]



Figure 1.2 Pods of *Mucuna pruriens* plant



Figure 1.3 Seeds of *Mucuna pruriens*

Some of the Ayurvedic properties of *Mucuna pruriens* are given below. ^[4]

Rasa	-	Madhura, Tikta
Guna	-	Guru, Snigdha
Veerya	-	Ushna
Vipaka	-	Madhura.
Prabhava	-	Shukrala, Vajikara
Rogagnata	-	Klaibya, Krimi, Yonishaithilya, Daurbalya
Karma	-	Brinhana, Vajikara, Vrishya, Balya, Vatahari, Krimighna, Mootrala

1.1.1 Medicinal properties of *Mucuna pruriens* seeds^[4]

Some of the medicinal properties of *Mucuna pruriens* seeds are stated below.

Astringent

Laxative

Anathematic

Alexipharmic and tonic

Diuretic

Anti-helmenthic

Aphrodisiac

Strengthens the body

Increase virility

Used in gynaecological disorders