



## *In vitro* Physicochemical, Phytochemical, Antimicrobial and Antidiabetic studies on *Mucuna pruriens* (Linn.) DC seeds

Srilakshmi Kusuma\*<sup>1</sup>, O. Aniel Kumar<sup>1</sup>, K. Lakshmi Narayana<sup>1</sup>, Sudhakar Pola<sup>2</sup>, K. Venkata Reddy<sup>3</sup>

<sup>1</sup>Department of Botany, College of Science & Technology, Andhra University, Visakhapatnam - 530 003, Andhra Pradesh, India.

<sup>2</sup>Department of Biotechnology, College of Science & Technology, Andhra University, Visakhapatnam - 530 003, Andhra Pradesh, India.

<sup>3</sup>Department of Biotechnology, SKSD Mahila Kalasala UG & PG (Autonomous), Tanuku - 534 211, West Godavari District, Andhra Pradesh, India.

**Received:** March 01, 2016; **Revised:** March 23, 2016; **Accepted:** April 15, 2016

**Abstract:** Diabetes is one of the most common endocrine diseases characterized by hyperglycemia due to absolute or relative deficiency of insulin which is currently affecting the citizens of both developed and developing countries. According to Williams textbook of endocrinology in 2013 it was estimated that over 382 million people throughout the world had diabetes. Plants have long been used for the treatment of diabetes, particularly in developing countries where most people have limited resources and do not have access to modern treatment. The presented study is aimed to evaluate the *Mucuna pruriens* seed extracts for its *in vitro* physicochemical, phytochemical, antioxidant and anti-diabetic studies. From the studies different solvent extracts of hexane and chloroform showed little or no activity on all assays performed whereas methanol extract of *Mucuna pruriens* showed significant bio properties. The preliminary studies of this plant crude methanol extract exhibited maximum compounds, hence the methanol extracts have under taken for its alpha amylase and alpha glucosidase inhibition activity. On the basis of the results obtained in the present investigation, it can be concluded that methanol extract of *Mucuna pruriens* had significant bioactive properties and may provide a support to use of the plant in traditional medicine for the management of diabetes.

**Key words:** *Mucuna pruriens*; diabetes; hyperglycemia; amylase; glucosidase inhibition

### Introduction

Diabetes, often referred as diabetes mellitus, a group of metabolic diseases in which the effected person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. Patients with high blood sugar will typically experience polyuria (frequent urination), they will become increasingly thirsty (polydipsia) and hungry (polyphagia). [Ann M. *et al.*, 2005]. Diabetes is a common and very prevalent disease affecting the citizens of both developed and developing countries. Diabetes mellitus involves a group of metabolic alterations resulting from defects in insulin secretion, action or both. It is of two types: Type I and Type II. Type I diabetes referred as juvenile diabetes, and is insulin dependent and known to affect only 5% of the diabetic population. The Type II, which is non-insulin dependent, usually develops in adults over the age of 40. It has already been established that chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels [Huang THW, *et al.*, 2005]. It has an adverse effect on carbohydrate, lipid and protein metabolism resulting in chronic hyperglycemia and abnormality of lipid profile. These lead to series of secondary complications including polyurea, polyphasia, ketosis, retinopathy as well as cardiovascular disorder [Kumar and Clark, 2002].

Diabetes mellitus is mainly caused by the abnormality of glucose metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin [Maiti R *et al.*, 2004].

A therapeutic approach for treating diabetes is to decrease postprandial hyperglycemia. This can be achieved through the inhibition of glucose hydrolyzing enzymes such as alpha glucosidase and alpha amylase [Kim *et al.*, 2002]. Alpha amylase and glucosidase inhibitors are drug-design targets in the development of compounds for the treatment of diabetes, obesity and hyperlipaemia [Franco *et al.*, 2002].

Medicinal plants have long been used since ancient times for the treatment of diabetes, particularly in developing countries where most people have limited resources and do not have access to modern treatment. Because of the possible importance of these inhibitors in plant physiology and animal and human nutrition, extensive research has been conducted on their properties and biological effects [Garcia-Olmedo F, *et al.*, 1987]. As per ethno botanical information referred that more than 800 plants are used for the treatment of diabetes throughout the world [Pushparaj *et al.*, 2000] but still there is an insufficient scientific evidences available of their antidiabetic activity [Bhosale and Hallale, 2011].

### \*Corresponding Author:

Srilakshmi Kusuma,

Lecturer in Botany,

SVKP & Dr.K.S.Raju Arts and Science College,

Penugonda, West Godavari District,

Andhra Pradesh, India.

*Mucuna pruriens* Linn. belongs to the family Fabaceae, it is popularly known as “Magic bean” in traditional Indian system of medicine. This plant is widely distributed in South East Asia largely found in Bangladesh, India, Sri Lanka, Malaysia and also found in Asia, America and Africa [Fung *et al.*, 2011]. All parts of *M. Pruriens* are generally used to treat diabetes mellitus [Bhaskar *et al.*, 2008] and cancer [Sathiyarayanan and Arulmozhi, 2007] whereas the seeds have multi-diversified functions like free radical mediated diseases management, rheumatoid arthritis, diabetes, atherosclerosis, nervous disorders, analgesic, antipyretic activity and in the management of Parkinsonism [Bhaskar *et al.*, 2011]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [Duke AT. 1995]. The scientific knowledge and pharmacognosy and biological activity of the plant *Mucuna pruriens* is least explored. Hence the present study was investigated in details for its evaluated for its Physicochemical, phytochemical, antioxidant and in vitro  $\alpha$ -glucosidase and  $\alpha$ -amylase enzyme inhibition activities.

## Materials and Methods

### Study area

Presented study was conducted in Visakhapatnam District, Andhra Pradesh, India.

### Collection of plant materials

Seeds of *Mucuna pruriens* were collected from the local supplier and were assessed biologically by the faculty members of Department of Botany.

### Preparation of plant extracts

The seeds of *Mucuna pruriens* were dried in shade and powdered in a mechanical grinder. The coarsely powdered material weighed and extracted successively with Hexane, Chloroform and Methanol using a soxhlet extractor for five to six hours at temperature not exceeding the boiling point of the solvent. For each 100 grams of dry material 2 litres of solvent was used. The extracted solvents were concentrated under reduced pressure (in vacuo at 40°C) using a rotary evaporator. The residue obtained were designated as crude extracts and stored in a freezer at -20°C until assayed. All the extracts were subjected to qualitative and Quantitative chemical investigation (Kokate CK, 2005). Details of various tests performed for the presence of physicochemical and phytoconstituents in the extracts are as under.

### Physicochemical parameters

The physico-chemical parameters were used to analyze the purity and quality of the extract. The extracts were evaluated for parameters like loss on drying, ash content and different extractive values. Ash value is useful in determining authenticity and purity of drugs and also these values are important for quantitative standards. Studied extracts were

evaluated by following physicochemical parameters were given below.

### Extractive values

Extractive values determine amount of active constituents present in given plant material in given solvent. Extracts were prepared with various solvents by standard methods. Percentage of dry extract was calculated in terms of air-dried powder. The results of extractive value are given in table 2.

### Loss on Drying (LOD)

Loss on drying is the loss in weight in percent w/w resulting from loss of water and volatile matter of any kind that can be driven off under specific conditions. 5 gm of air-dried plant material (extract) was placed in a crucible of silica. The extract was spread in a thin uniform layer. The crucible was then placed in the oven at 105°C. The extract was dried for 4 hours and cooled in a desiccator to room temperature and weight of the cooled crucible plus powder was noted.

### Determination of ash content

For the determination of ash, clean empty crucible was placed in a muffle furnace at 600°C for an hour, cooled in desiccator and then weight of empty crucible was noted (W1). One gram of each of the medicinal plant powder was taken in crucible (W2). This was then ignited over a burner with the help of blowpipe, until it is charred. Then the crucible was placed in muffle furnace at 550°C for 2-4 hour. The appearances of gray white ash indicate complete oxidation of all organic matter in the medicinal plant material. After ashing furnace was switched off. The crucible was cooled and weighed (W3). Percent ash was calculated by following formula:

$$\% \text{ Ash} = \frac{\text{Difference in weight of Ash}}{\text{Weight of the medicinal plant powder}} \times 100$$

Difference in weight of ash = W3 - W1

### Preliminary phytochemical investigation

The plant seed extract was taken in a test tube and distilled water was added to it such that plant powder soaked in it and shaken well. The solution then filtered with the help of filter paper and filtered extract of the selected plant samples were taken and used for further phytochemical analysis. Phytochemical screening of all the selected medicinal plants was qualitatively tested for the presence of chemical constituents.

The extracts of phytochemical analysis for identification of bioactive chemical constituents were carried out by using standard methods of Sofowora, Trease & Evans, Kokate, Harbone and Raman (Sofowora A, 1993; Trease GE & Evans WC, 1994; Kokate CK, 2005; Harbone JB, 1984 and Raman N, 2006).

**Detection of Alkaloids**

**Mayer's Test:** Plant extracts were treated with Mayer's reagent (1.36 mercuric chloride and 5gms of potassium iodide was dissolved in 100ml distilled H<sub>2</sub>O). The formation of yellow cream precipitate indicates the presence of alkaloids.

**Wagner's Test:** Plant extracts were treated with Wagner's reagent (1.27g iodine, 2gm potassium iodide in 100ml distilled H<sub>2</sub>O). The formation of brown or reddish precipitate indicates the presence of alkaloids.

**Detection of Cardiac Glycosides**

**Kella – Killani Test:** Plant extract was dissolved in glacial acetic acid containing traces of FeCl<sub>3</sub>. Then the tube was held at an angle of 45°, 1ml of conc. H<sub>2</sub>SO<sub>4</sub> was added down the side purple ring at the interface indicates cardiac glycosides.

**Detection of Terpenoids**

**Salkowski Test:** To the 1ml of plant extract, 2ml of chloroform was added. Then 3ml of conc. H<sub>2</sub>SO<sub>4</sub> was added carefully to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

**Detection of Steroids**

2ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2ml of H<sub>2</sub>SO<sub>4</sub>. The colour changes from violet to blue or green indicates the presence of steroids.

**Detection of Flavonoids**

**Ferric chloride Test:** Test solution when treated with few drops of FeCl<sub>3</sub> would result in the formation of blackish red colour indicating the presence of flavonoids.

**Alkaline Reagent Test:** Plant extracts were treated with few drops of NaOH solution. Formulation of intense yellow colour, which becomes color less on addition of dilute acid indicates the presence of flavonoids.

**Lead Acetate Test:** Plant extracts were treated with few drops of lead acetate solution. Formation of a yellow precipitate indicates the presence of flavanoids.

**Detection of Tannins**

To the 1ml of plant extract, few drops of 1% FeCl<sub>3</sub> solution were added. The appearance of blue, black, green or blue green precipitate indicates the presence of tannins.

**Anthraquinones:**

About 0.5 g of each extract was boiled with 10 % HCl for few minutes in water bath, filtered and allowed to cool. Equal volume of CHCl<sub>3</sub> was added to the filtrates. Few drops of 10% ammonia was

added to the mixtures and heated. Formation of rose-pink color indicated the presence of anthraquinones.

**Detection of Saponins**

**Foam Test:** About 2ml of distilled H<sub>2</sub>O and 1ml of plant extract were mixed and shaken vigorously. A stable persistent froth indicates the presence of Saponins.

**Froth Test:** Plant extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15minutes. Formation of 1cm layer of foam, which is stable for 15 minutes indicates the presence of Saponins.

**Phlobatanins:** The extracts (0.5 g) were dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Precipitate shows the absence of phlobatanins.

**Reducing Sugars:** The extracts were shaken with distilled water, filtered and boiled with few drops of Fehling's solution A and B for few minutes. No orange/red color indicates the absence of reducing sugars.

**Antibacterial Assays**

**Bacterial Isolation and Maintenance:** Blood samples were taken from patients with clinical positive cases and samples were transferred in culture bottles of nutrient broth (Hi Media, Mumbai, India). Bottles were incubated at 36.7°C for 7 days. Bottles showing positive growth index were Gram stained and sub cultured on nutrient agar plates, These plates were aerobically incubated for 24-48 h at 37° in B.O.D. All bacterial pathogens were identified by standard microbiological and biochemical procedures. These biochemical tests include carbohydrates fermentation test, urease test, oxidase test, haemolysis of blood, catalase test, motility test and growth of pathogens on specific media etc. [Shimeld LA. 1998; Hawkey P, Law D, 2004].

**Determination of Antibacterial activity**

Bacterial inoculums were prepared from overnight grown cultures (24 h) in peptone water (Hi Media, Mumbai, India), and the turbidity was adjusted equivalent to 0.5 McFarland units (approximately 10<sup>8</sup> CFU/ml).

The crude extracts of different plants were subjected to antibacterial assay using the Agar Well Diffusion Method of Murray *et al.*, modified by Olurinola P.F. 20ml of nutrient agar was dispensed into sterile universal bottles these were then inoculated with 0.2ml of cultures mixed gently and poured into sterile petridishes. After setting a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make four uniform wells in each petridish. A drop of molten nutrient agar was used to

seal the base of each cup. To test the antibacterial activity all extracts were dissolved in DMSO to make a final concentration of 100mg/ml and the wells are filled with 20µl of the extract and allow diffusing for 45 minutes. These plates were incubated for a period of 24 h at 37°C in incubator. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates. The average value of inhibition and standard deviation were calculated. The zone of inhibition was compared with that of standard ciproflaxacin 10µg/1000µl.

### Antibacterial Assays

#### Fungal isolation and maintenance

Swab samples from patients were taken by gently rubbing a sterile cotton swab over the infected area and were incubated in saboured dextrose broth for primary isolation of the pathogens. These plates were then aerobically incubated for 24-72 h at 30°C in B.O.D. incubator for fungal species isolation. When growth was appeared on cultured plates, fungal pathogens were identified on the basis of carbohydrate assimilation or fermentation, their macroscopic and microscopic morphologic features after growth on specialized media. Moulds are identified primarily on the basis of their macroscopic and microscopic morphologic features.

#### Assay for antifungal activity

Fungal inoculums were prepared from overnight grown cultures (24 h) in saboured dextrose broth (HiMedia, Mumbai, India), and the turbidity was adjusted equivalent to 0.5 McFarland units (equivalent to  $1.5 \times 10^5$  or  $10^6$  CFU/ml). Flucanazole (10µg/ml) was taken as the positive control for antifungal activity. The DMSO was taken as negative control to determine possible inhibitory activity of the dilutant extract. The susceptibilities of the isolated pathogens were determined by using agar well diffusion method. 20ml of sabouraud dextrose agar (SDA) was taken into sterile universal bottles and these were then inoculated with 0.2ml of overnight culture of the test organism, mixed gently and poured into sterile petridishes. After setting wells of 6mm was punctured with the help of a sterilized cork borer into the pre-solidified sabouraud dextrose agar plates containing the test organism. Using the micropipette, 40 µl of each extract was poured into the different wells of the inoculated plates. Standard antifungal was used as positive control and fungal plates was incubated at 37°C for 72 h. The diameter of zone of inhibition was measured. Each experiment was done in triplicate and mean values were taken. Antifungal activity was measured by the diameter (mm) of the clear inhibitory zone formed around the well.

### Antidiabetic studies

**α-Amylase Inhibition Assay:** Inhibition of α-amylase was carried out using the pre incubation method described by Geethalakshmi *et al.*, 2010.

Briefly, porcine pancreatic α-amylase (Sigma) was dissolved in ice-cold distilled water (5 unit/ml solution). Potato starch (1% w/v) in 20mM phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride, was used as the substrate solution. Plant extract (40µl) was mixed with 40µl porcine pancreatic α-amylase and 80µl of 20 mM phosphate buffered saline (pH 6.9). Tubes were pre incubated for 15 min at 37°C and then 1% potato starch (40µl) was added to all the tubes. Final concentration of plant extract used during screening was 1 mg/ml. Series of final concentrations used for the extracts with higher inhibitory effects included 1, 2.5, 5 and 10 mg/ml. Control was carried out in the absence of plant extract or standard inhibitor. Test blanks were conducted in the presence of plant extracts without α-amylase. A blank reaction was carried out with 40µl methanol replacing the plant extract. Acarbose (Sigma) (200µg/ml) was used as the standard inhibitor. Reaction mixtures were incubated for 15 min at 37°C. Dinitrosalicylic acid colour reagent (96 mM 3, 5-dinitrosalicylic acid, 5.31 M sodium potassium tartarate in 2 M NaOH) was added (100 µl) to all the tubes and was kept immediately in a water bath at 85°C for 15 min. Distilled water (900 µl) was added to each tube and the absorbance was measured at 540 nm.

#### Calculation of Percentage Inhibition of Enzyme

**Activity:** Percentage inhibition was calculated using the following formula.

$$\left\{ \frac{(\text{Absorbance of Test} - \text{Absorbance of Test Blank}) \times 100}{(\text{Absorbance of Control} - \text{Absorbance of Control Blank})} \right\} \text{Percentage inhibition}$$

**α-Glucosidase Inhibition Assay:** The α-glucosidase inhibition was determined using the method as described by Elya *et al.*, 2012. Briefly, 200 µl of 67 mM sodium phosphate buffer (pH 6.8) and 120 µl of 10 mM *p*-Nitrophenyl α-D- Glucopyranoside (Sigma) was added to tests, control and the blanks. Plant extract (40µl) was added to the test and test blank. The mixtures were pre incubated for 15 min at 37°C. After incubation, 40µl of 0.1 U α-glucosidase from *Saccharomyces cerevisiae* (Sigma) was added to the tests and control. Final concentration of plant extract used during screening was 0.5 mg/ml. Series of final concentrations used for the extracts with higher inhibitory effects included 1, 2.5, 5 and 10 mg/ml. The reaction mixture was incubated for 15 min at 37°C. Reaction was terminated by adding 200 mM sodium carbonate (800 µl). The hydrolysis of α-D-glucopyranoside to *p*-nitrophenol was measured at 405 nm. Acarbose (200 µg/ml) was used as the standard inhibitor.

#### Calculation of IC<sub>50</sub>

The concentration of the extract that inhibits 50% of the enzyme activity (IC<sub>50</sub>) was calculated. Extracts with high inhibitory activity were analyzed using a

series of suitable extract concentrations. IC50 values were determined by plotting percent inhibition (Y axis) versus log10 extract concentration (X axis) and calculated by logarithmic regression analysis from the mean inhibitory values [Sudha P, *et al.*, 2011].

### Statistical Analysis

All experiments were performed three times. Each experiment was carried out in triplicates. Data are expressed as mean  $\pm$  standard deviation. Statistical analysis was performed using ANOVA. Values of p which were  $<0.05$  were considered as significant.

### Results and Discussion

The phytochemical constituents of the *Mucuna pruriens* (Linn.) DC seeds hexane, chloroform and methanol extracts were shown in Table 1. From the

phytochemical screening, alkaloids, glycosides, terpenoids, saponins, tannins and reducing sugars were detected. Steroids, flavonoids, anthraquinones, phlobatanins and saponins were not detected in methanol extract of *Mucuna* seeds. Flavonoids were present in hexane extract of seeds of *Mucuna pruriens* and steroids detected in chloroform extract of *Mucuna pruriens*. All the extracts showed some degree of antimicrobial activity, it was significant in *Mucuna pruriens* methanol extract (ZI= 27mm). The extract of *Mucuna bracteata* seeds was effective against *Escherichia coli*. The zone of inhibition against all microorganism in detail was represented in table 4. Antibiotics of Ciprofloxacin (Bacteria) and Fluconazole (Fungi) were used at 10 $\mu$ g/ml, respectively.

**Table 1:** Phytochemical content of the plant (seeds) extracts

Chemical Components	Hexane Extract	Chloroform Extract	Methanol Extract
Alkaloids	-	+	+
Glycosides	-	-	+
Terpenoids	+	-	+
Steroids	+	+	-
Flavonoids	+	-	-
Tannins	-	+	+
Anthraquinones	-	-	-
Saponins	+	+	-
Phlobatanins	-	-	-
Reducing sugars	-	+	+

Key: + = detected; - = not detected

**Table 2:** Physiochemical analysis of *Mucuna pruriens* L. extract

Parameters	Observations
Description	Yellowish brown color
Extractive Value	83.23%
Loss on Drying (%) at 105°C	2.72%
Ash content (%)	3.78%

**Table 3:** Quantitative analysis of phytochemical constituents *M. pruriens*

Phytochemical compounds	<i>Mucuna pruriens</i> g/100g
Phenolics	3.823 $\pm$ 0.25
Tannins	0.296 $\pm$ 0.56
Flavanoids	0.373 $\pm$ 0.034
Alkaloids	1.630 $\pm$ 0.068
Saponins	0.616 $\pm$ 0.013

All the values are determining triplicates mean  $\pm$  Standard Error; a- Gallic acid equivalent; b-Tannic acid equivalent; c-Quercetin equivalent; d - Colchicines equivalent's

**Table 4:** Antimicrobial activity of *M. pruriens* by well diffusion method

Microorganisms	Hexane	Chloroform	Methanol	DMSO control	Positive control
<i>E. coli</i>	7	10	17	-	20
<i>P. vulgaris</i>	10	9	12	-	27
<i>K.pneumoniae</i>	7	7	14	-	31
<i>S.aureus</i>	10	9	10	-	17
<i>C.albicans</i>	8	10	12	-	24
<i>A.niger</i>	11	9	15	-	17
<i>E. floccosum</i>	9	10	11	-	16
<i>T.mentagrophytus</i>	7	8	13	-	21

Concentration of extract- 100 $\mu$ l/well, (-) - No zone of inhibition observed, Positive controls - Bacteria (Ciprofloxacin) Fungi (Fluconazole) 10 $\mu$ g/ml, Solvent control - DMSO.

The seeds of *M. pruriens* was successively extracted with different solvents of increasing polarity (Hexane, Chloroform and Methanol). The preliminary phytochemical screening of methanol extract showed presence of phytochemicals of phenols, flavonoids, tannins, steroids, triterpenoids and glycosides. Hence the methanolic extract of *M. pruriens* assayed for *in vitro* alpha amylase and alpha glucosidase inhibitory activity. Four different concentration of 1, 2.5, 5 and 10 mg/ml were tested, the extract showed good inhibitory effect at all the tested concentrations was 10mg/ml the maximum inhibitory effect of methanol extract showed significant alpha amylase and alpha glucosidase inhibitory activity (69% and 87%) with IC<sub>50</sub> value of 7.8mg and 6.1mg respectively.

**Table 5:** Percent of inhibition and IC<sub>50</sub> values of  $\alpha$ -amylase at 60mg/ml

Plant Name	Solvent type	% Inhibition	IC <sub>50</sub> ( $\mu$ g/ml)
<i>Mucuna pruriens</i> (Linn.) DC	Hexane	43.27 $\pm$ 0.2	33.10 $\pm$ 0.22
	Chloroform	51.05 $\pm$ 1.2	27.35 $\pm$ 1.04
	Methanol	69.23 $\pm$ 0.1	7.89 $\pm$ 1.04

IC<sub>50</sub> = concentration of extract with 50% of  $\alpha$ -amylase inhibitory activity

Note: n.d. = not determined

**Table 6:** Percent of inhibition and IC<sub>50</sub> values of  $\alpha$ -glucosidase at 60mg/ml

Plant Name	Solvent type	% Inhibition	IC <sub>50</sub> ( $\mu$ g/ml)
<i>Mucuna pruriens</i> (Linn.) DC	Hexane	30.18 $\pm$ 1.1	11.05 $\pm$ 0.33
	Chloroform	65.32 $\pm$ 2.1	12.56 $\pm$ 3.1
	Methanol	87.64 $\pm$ 2.5	6.12 $\pm$ 1.01

IC<sub>50</sub> = concentration of extract with 50% of  $\alpha$ -glucosidase inhibitory activity

Note: n.d. = not determined S.A: Standard acarbose

## Discussion

In current scenario throughout the world there were 171 million diabetic cases were reported in 2000 and were expected to be raising 366 million on 2030 [Si M.M *et al.*, 2010]. Two key enzymes play critical role in patients with diabetes these enzymes are  $\alpha$  – glucosidase and  $\alpha$ -amylase [Ali *et al.*, 2006; Karthic *et al.*, 2008]. Many side effects are associated with Commonly used synthetic anti-oxidants like Butylated hydroxyanisole (BHA) and the related compound butylated hydroxytoluene (BHT) that are used in food industry as preservatives [Kommu S *et al.*, 2011] possesses many side effects [Debasmita M *et al.*, 2012].

In India there are numerous herbal extracts has been reported for their anti-diabetic properties and are being used in traditional Indian medicine for the treatment of diabetes. Therefore, present research is attracted more attention. In this study, Physicochemical, Phytochemical, Antimicrobial and Antidiabetic Studies were conducted using successive solvent extracts of hexane, chloroform and methanol of *Mucuna Pruriens* (Linn.) DC seeds, from the reports methanol extracts proved and the same has

been supported the reports as anti-diabetic principles by various researchers [Reher G. *et al.*, 1991; Shimizu M, *et al.*, 1984; Ivorra *et al.*, 1989].

The medicinal plants or natural products involve retarding the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes. Several  $\alpha$ -amylase inhibitors including acarbose, voglibose and miglitol are clinically used for treatment but their prices are high and clinical side effects occur [Scott and Spencer, 2000]. Hence screening of  $\alpha$ -glucosidase inhibitors from plants and synthetic sources is increasing and inhibitors of these enzymes have been recently developed from natural sources [Jung M. 2006].

In this study, *in vitro* effect of different concentrations of various solvent extracts of *M. pruriens* seeds was evaluated. At the concentration the 10mg/ml of plant extract showed significant inhibitory activity. The present study indicated that *M. pruriens* seeds could be useful in the management of postprandial hyperglycemia.

## Conclusion

In this present study *M. pruriens* seeds contained many bioactive chemical constituents including glycosides, terpenoids, alkaloids, saponins, tannins and reducing sugars. The present study clearly exhibited that the methanol extracts of seeds *Mucuna pruriens* were found to be more effective in assays performed compared to hexane and chloroform extracts. On the basis of the results obtained in the present study, it can be concluded that methanol extract of *Mucuna pruriens* seeds had significant bioactive properties and may provide a support to use of the plant in traditional medicine. *In vitro* alpha amylase and alpha glucosidase activity of crude methanol extract of also proved that *M. pruriens* seed extract might be used as alternatives for the management and control of Diabetes. Further studies in isolation, purification and characterization which is responsible for inhibiting activity, has to be done for the usage of antidiabetic agent.

## References

1. Al-Dabbas MM, Kitahara K, Suganuma T, Hashimoto F, Tadera K. Antioxidant and alpha-amylase inhibitory compounds from aerial parts of *Varthemiaiphionoides* Boiss. *Biosci Biotechnol Biochem.* 2006;70 (9): 2178–2184.
2. Ali H, Houghton P.J, Soumyanath A, J. *Ethnopharmacol*, 2006, 107, 449.
3. Karthic K, Kirthiram K.S, Sadasivam A, Thayumanavan B. *Indian J Exp Biol*, 2008, 46, 677.

4. Ann M. Aring, David E. Jones, James M (2005) Falko Evaluation and Prevention of Diabetic Neuropathy. *Am Fam Physician* 71: 2123-2128.
5. Bhaskar A, Nithya V, Vidhya VG. Phytochemical evaluation by GC-MS and antihyperglycemic activity of *Mucuna pruriens* on Streptozotocin induced diabetes in rats. *Journal of Chemical and Pharmaceutical Research* 2011; 3(5): 689- 696.
6. Bhaskar A, Vidya VG, Ramya M. Hypoglycemic effect of *Mucuna pruriens* seed extract on normal and streptozotocin-diabetic rats. *Fitoterapia* 2008; 79: 539-543.
7. Bhosale U. P, Hallale B. V, *Asian J Plant Sci*, 2011, 1, 96.
8. Debasmita M, Mala M, *Asian J Plant Sci*, 2012, 2,102.
9. Duke AT. *Handbook of Medicinal Herbs*. 3rd Edn. CRS, Press, London 1995; pp: 220.
10. Elya B, Basah K, Munim A, Yuliastuti W, Bangun A, Septiana EK. Screening of  $\alpha$ -glucosidase inhibitory activity from some plants of Apocynaceae, Clusiaceae, Euphorbiaceae, and Rubiaceae. *Journal of Biomedicine and Biotechnology*; 2012.
11. Franco O.L, Rigden D.J, Melo F.R, Grosside-Sa M.F, *Eur J Biochem*, 2002, 269,397.
12. Fung SY, Tan NH, Sim SM, Marinello E, Guerranti, Aguiyi JC. *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* 2011; 49: 254-259.
13. Garcia-Olmedo F, Salcedo G, Sanchez-Monge R, Gomez L, Royo J, Carbonero P, *Cell Biol*, 1987, 4, 275.
14. Geethalakshmi R, Sarada DVL, Marimuthu P, Ramasamy K.  $\alpha$ -Amylase inhibitory activity of *Trianthema decandra* L. *International Journal of Biotechnology and Biochemistry*. 2010;6(3):369-376.
15. Harborne, J.B. *Phytochemical Methods; A guide to Modern Technique of Plant Analysis*, 2nd ed. Chapman, 1984, 100-101.
16. Hawkey, P. and Law, D. *Medical Bacteriology*. 2. Oxford University Press, New York, 2004.
17. Huang THW, Peng G, Kota BP, Li GQ, Yamahara J, Roufogalis BD *et al.*, Anti-diabetic action of *Punica granatum* flower extract: activation of PPAR-c and identification of an active component. *Toxicol App Pharmacol* 2005; 207:160-169.
18. Jung M, Park M, Chul H.L, Kang Y, Seok-Kang E, Ki-Kim S, *Curr Med Chem*, 2006, 13, 1.
19. Kim Y.M, Jeong Y.K, Wang M.H, Lee W.Y, Rhee H.I, *Nutrition*, 2005; 21, 756. M. B, Ajimire P. V, Wagh A. E, Mohan M, Shivashanmugam A.T, *Asian J Plant Sci*, 2011, 1,101. [4] Franco O.L, Rigden D.J, Melo F.R, Grosside-Sa M.F, *Eur J Biochem*, 2002, 269,397.
20. Kokate, C.K. *Practical pharmacognosy*. Published by Jain, M.K. for Vallabh Prakashan, Pitampura, New Delhi, 2005, p. 107.
21. Kommu S, Chiluka V. L, Shankar G, Matsyagiri N. L, Shankar L. M, Sandhy S, *Der Pharmacia Sinica*, 2011, 2,193.
22. Kumar A, Rajput G, Dhatwalia VK, Srivastav G. Phytocontent Screening of *Mucuna* Seeds and Exploit in Opposition to Pathogenic Microbes. *Journal of Biological & Environmental Sciences* 2009, 3(9): 71-76.
23. Kumar P, Clark M. *Diabetes Mellitus and Other Disorders of Metabolism*. In: *Clinical Medicine*, Sanders, WB (Eds.). 2nd ed. Elsevier, London; 2002, 1069-1071.
24. Maiti R, Jana D, Das UK, Ghosh D. Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin induced diabetic rats. *J Ethnopharmacol* 2004; 92: 85-91.
25. Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Tenover, H.R. *Manual of Clinical Microbiology* 6th edition, ASM Press, Washington, DC. 1995, 15-18.
26. Prashant A.B, Bhanudas K.S, *Asian J Plant Sci*, 2011, 1, 91.
27. Pushparaj P, Tan CH, Tan BKH, J. *Ethnopharmacol*, 2000, 72, 69.
28. Raman, N. *Phytochemical Techniques*. New Indian Publishing Agencies, New Delhi, 2006, 19- 20.
29. Reher G, Slijepcevic M, Krans L, *Planta Med*, 1991, 57, 57.; Schimizu M, Ito T, Rshima S, Mayashi T, Arisawa M, Morita-Kurokawa S, *Phyto chem*, 1984, 23, 1885.
30. Ivorra M.D, Paya M, Villar A, J. *Ethnopharmacol*, 1989, 27, 243.

31. Sathiyarayanan L, Arulmozhi S. *Mucuna pruriens* Linn. A comprehensive review. *Pharmacology Review* 2007; 1: 157-162.
32. Scott L.J, Spencer C.M, *Drugs*, 2000, 59, 521.
33. Sharma P, Mehta S.C, Dubey G, Lakshmayya B, Kaushik S, *Der Pharmacia Sinica*, 2011, 2,99.
34. Shimeld, L.A. *Essential of Diagnostic Microbiology*. International Thomas Publishing Company, USA, 1998.
35. Si M.M, Lou J.S, Zhou C.X, Shen J.N, Wu H.H, yang B, J. *Ethnopharmacol*, 2010, 128,154.
36. Sofowora, A. *Medicinal plants and Traditional Medicine in Africa*. Spectrum Books Ltd. (Pub.), Ibadan, 17, 1993, 1-153.
37. Subramanian R, Asmawi M.Z, Sadikun A, *Acta Biochim Pol*, 2008, 55, 391.
38. Sudha P, Zinjarde SS, Bhargava SY, Kumar AR. Potent  $\alpha$ -amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC Complementary and Alternative Medicine*; 2011.
39. Trease, G.E. and Evans, W.C. *Pharmacognosy*. Bailliere Tindall, London, 18, 1989, 45-50.

**Cite this article as:**

Srilakshmi Kusuma, O. Aniel Kumar, K. Lakshmi Narayana, Sudhakar Pola, K. Venkata Reddy. *In vitro* Physicochemical, Phytochemical, Antimicrobial and Antidiabetic studies on *Mucuna pruriens* (Linn.) DC seeds *International Journal of Bioassays* 5.6 (2016): 4650-4657.

**Source of support:** Nil  
**Conflict of interest:** None Declared