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STANDARDIZATION AND PHYTOCHEMICAL SCREENING OF *MUCUNA PRURIENS*

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ABSTRACT
Mucuna pruriens belongs to the family Fabaceae and it is commonly known as Velvet bean. *Mucuna pruriens* is the most popular drug in Ayurvedic system of medicine. Various parts of *Mucuna pruriens* are generally used for the treatment of impotence, diabetes mellitus**,** bone fractures, cough, dog bite, cancer and madness. The present study was designed for the standardization and phytochemical screening of various extracts of whole plant powder of *Mucuna pruriens.* Physicochemical parameters like loss on drying, determination of total ash, determination of pH range and acid insoluble ash of whole plant powder of *Mucuna pruriens* were estimated based on the methods recommended by World Health Organization. Standardization includes the estimation of physicochemical parameters such as loss on drying, determination of pH range, determination of total ash, determination of water soluble ash, determination of acid insoluble ash, determination of sulfated ash and determination of hot water & ethanol-extractable matter. Preliminary phytochemical screening and TLC studies for all the extracts like chloroform, ethyl acetate and methanol were carried out by standard methods. The result of physicochemical parameters values was found to be within the ranges and valuable to estimate the chemical constituents present in the crude drug. Report of phytochemical screening for all the extracts revealed the presence of various phytoconstituents like alkaloids, flavonoids, tannins and phenolic compounds in the leaves of *Mucuna pruriens* The generated information of the present study will provide data which is helpful in the correct identification & authentication of this medicinal plant and may help in preventing its adulteration.

KEYWORDS: Mucuna Pruriens, Standardization, Phytoconstituents, Adulteration.

INTRODUCTION
Plants have always been a part of medicinal science from the beginning of human civilization to the present modern world of synthetic medicines. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter.[1] Traditionally *Mucuna pruriens* is used in treating diseases such as impotence, diabetes mellitus, diarrhoea, dysentery and cancer[2] Ethnomedical information of *Mucuna pruriens* shows that root is used to treatment for blood purifier, diuretic and for treating gout and kidney stones.[3] In Brazil the plant is used as anthelmintic, aphrodisiac, diuretic, hydropsy, intestinal worms and nerve tonic. The leaves part is used as aphrodisiac, uterine stimulant and in dysentery.[4] Seeds of *Mucuna pruriens* is used to cure impotency and used in diseases such as diarrhea, diabetes, gonorrhea, muscular pain, persistent coughs, pulmonary tuberculosis, rheumatic disorders, snake bite and worm infestation.[4] *In vitro* assays indicated that a whole plant of ethyl acetate and methanol extracts of *M. pruriens*, containing large amounts of phenolic compounds, exhibited high antioxidant and free radical scavenging activities.[5] Standardization is useful for determining crude drugs & it gives an idea about the nature of the chemical constituents present.[6] Standardization can show a vital role in the estimation of herbal drugs and can also become a significant quality control method as well as for suitable stability testing of the product.[6] Standardization of herbal formulations is essential in order to evaluate the quality of drugs, based on the concentration of their active principles. The traditional approach towards standardization is insufficient for current herbal market and hence there is need for more advanced techniques for standardization. Phytoconstituents play an important role in the field of new drugs research and development because of their low toxicity, easy availability and cost effective. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Flavonoids are important group of polyphenols widely distributed among the plant flora. Structurally, they are made of more than one benzene ring in its structure (a range of C15 aromatic compounds) and numerous reports support their use as antioxidants or

free radical scavengers. The objective of the current study was to identify, authenticate and prevent the adulteration of the plant *Mucuna pruriens.*

MATERIALS AND METHODS

Collection and authentication of plant material

The whole plant of *Mucuna pruriens* (Fabaceae) was collected in the month of April from botanical garden, Hanamkonda, Warangal district. It was shade dried away from sunlight and stored suitably. The plant material was taxonomically identified by Dr. K. Raju, Assistant Professor, Department of Botany, Kakatiya University, Warangal district, Telangana, India and a voucher specimen was deposited in the herbarium against accession number 4612 for future reference.

Extraction

The freshly collected plant material of *Mucuna pruriens* was shade dried and pulverized to get a coarse powder with a mechanical grinder. A weighed quantity of the powder was passed through Sieve no. 40 which was stored in an air tight container for further use. Powder of *Mucuna pruriens* was individually extracted with chloroform, ethyl acetate and methanol by continuous soxhlet extraction method. The solvents were removed by rotary vacuum evaporator, the remaining mass of extracts were concentrated and dried. The extracts were stored in desiccator for further studies.

Standardization

Loss on drying / Moisture content

About 4.0 grams of *Mucuna pruriens* plant powder was placed, in an accurately weighed moisture disc. For estimation of loss on drying, it was dried at 103°C for 4 hours in an oven, then it was cooled in a desiccator for 35 minutes, and it is weighed without delay. The loss of weight was calculated as the content in mg per g of air-dried material.

Determination of total ash

Mucuna pruriens plant powder (3 gm) was placed in a previously ignited (250°C for 2 hrs) and tarred crucible accurately weighed. Dried material was spread in an even layer in the crucible and the material was ignited by gradually increasing the temperature to 350°C for 3 hours in a muffle furnace (Nabertherm) until it is white, indicating the absence of carbon. Then it was cooled in a desiccator and weighed. Total ash content was calculated in mg per g of air-dried material.

Determination of water soluble ash

Twenty (20) ml of water was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 20 minutes. Insoluble matter was collected on an ash less filter paper. Then it was washed with hot water and ignited in a crucible for 15 minutes at a temperature of 380°C in a muffle furnace. The residue was allowed to cool in a suitable desiccator for 30 minutes, and then weighed without delay. The weight of the residue was subtracted in milligram from the weight of total ash. Water-soluble ash content was calculated as mg per g of air-dried material.

Determination of acid-insoluble ash: Twenty (20) ml of hydrochloric acid (70g/l) total solid content (TS) was added to the crucible containing the total ash. It was covered with a watch-glass and then boiled gently for 20 minutes. The watch-glass was rinsed with minute quantity of hot water and this liquid is added to the crucible. The insoluble matter was collected on an ash less filter-paper (Whatmann -41) and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, and then it was ignited by gradually increasing the heat to 400°C for 3 hours in a muffle furnace (Nabertherm) to constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes, and then weighed without delay. Acid-insoluble ash content was calculated as mg per g of air dried material.

Determination of sulfated ash

A suitable crucible (silica) ignited at 550°C to 600°C for 30 minutes and the crucible was cooled in a desiccator (silica gel) and was weighed it accurately. 1gram of the whole plant powder of the *Mucuna pruriens* was placed in a previously ignited crucible, ignited gently at first, until the substance was thoroughly white. The sample was cooled and moistened with a small amount (usually 1 ml) of sulfuric acid (1660 g/l) and heated gently at a temperature as low as practicable until the sample is thoroughly charred. After cooling, the residue was moistened with a small amount (usually 2 ml) of sulfuric acid (1660 g/l) and heated gently until white fumes were no longer evolved, and ignited at 600°C $\pm$ 25°C until the residue was completely incinerated. Ensured that the flames were not produced at any time during the procedure. The crucible was cooled in a desiccator (silica gel) and weighed accurately. This was repeated until the sample reaches a constant weight and the percentage of residue was calculated.

Determination of pH range: The pH of different formulations in 1% w/v (1g: 100ml) and 10% w/v (10g: 100ml) of water soluble portions of whole plant powder of *Mucuna pruriens* were determined using standard simple glass electrode pH meter.[7]

Determination of hot water and ethanol-extractable matter: About 3.0g of whole plant powder of *Mucuna pruriens*, was placed in an accurately weighed, glass stoppered conical flask. For estimation of hot water extractable matter, 50ml of distilled water was added to the flask and weighed to obtain the total weight including the flask. The contents were shaken well and allowed to stand for 50min. A reflux condenser was attached to the flask and boiled gently for 55 minutes; cooled and weighed. The flask was readjusted to the original total weight with distilled water and it was shaken well and filtered rapidly through a dry filter. Then the filtrate was

transferred to an accurately weighed, tarred flat-bottomed dish (Petri disc) and evaporated to dryness on a water-bath. Finally, it was dried at 100°C for 3 hours in an oven, and it was cooled in a desiccator for 30 minutes, and weighed without delay. Same procedure was followed using ethanol instead of distilled water to determine extractable matter in ethanol. The extractable matter was calculated as the content in mg per gm of air - dried material

Phytochemical Screening

The phytochemical screening was carried out by standard procedures.[8] Different extracts of *Mucuna pruriens* were subjected to preliminary phytochemical screening for the detection of various phytochemical constituents such as carbohydrates, proteins, amino acids, steroids, tannins, flavonoids, alkaloids and glycosides.

Several tests are as follows

1. Test for Alkaloids

Sample (chloroform, ethyl acetate and methanol extracts) was evaporated, to the residue dilute hydrochloric acid was added. It was shaken well and filtered. With filtrate following tests were performed.

- **Dragendorff's test**

Little amount of the sample was treated with the Dragendorff's reagent**,** the appearance of reddish brown precipitate indicated the presence of alkaloids.

- **Mayer's test**

Sample (2-3ml) was treated with few drops of Mayer's reagent. Appearance of white precipitate indicated the presence of alkaloids.

- **Hager's test**

Sample (2-3ml) was treated with Hager's reagent. Appearance of yellow precipitate indicated the presence of alkaloids.

- **Wagner's test**

Sample (2-3ml) was mixed with few drops of Wagner's reagent. Appearance of reddish brown precipitate indicated the presence of alkaloids.

2. Test for Proteins[9]

- **Biuret test (General test)**

Test sample (3ml) was mixed with 4% NaOH and few drops of 1% $Cuso_4$ solution were added. Violet or pink color not appeared.

- **Millon's test:** Test sample (3ml) was mixed with 5ml of million's reagent. White precipitate is formed. On warming precipitate turn's brick red or the precipitate dissolves giving red colored solution.

- **Xanthoprotein test for proteins containing tyrosine or tryptophan**

Test solution (3ml) was mixed with 1ml of conc sulphuric acid. White precipitate is formed. It was boiled then precipitate turned yellow. Ammonium hydroxide was added, finally precipitate turned orange.

3. Tests for Amino Acids[9]

- **Ninhydrin test (General test)**

Test sample 3ml and 3 drops of 5% ninhydrin solution were heated in boiling water for 10 mins. Purple color is not appeared.

4. Test for Steroids[9]

Sample 2ml was mixed with 2ml of conc Sulphuric acid, it was well shaken then chloroform layer was appeared red and acid layer has shown greenish yellow fluorescence.

- **Liebermann-Burchard reaction**

Sample (2ml) was mixed with chloroform. 1-2 ml of acetic anhydride was added and 2 drops conc. Sulphuric acid was added from the sides of the tube. First red then blue and finally green colour appeared.

- **Liebermann's reaction**

Sample (3ml) was mixed with 3ml of acetic anhydride. It was first heated and then cooled, later few drops of conc. Sulphuric acid were added and blue colour appeared.

5. Test for Glycosides[9]

Free content of the sugar extract was determined. The sample was hydrolyzed with mineral acid (dilute hydrochloric or dilute sulphuric acid). Again the total sugar content of the hydrolyzed extract was determined. Increase in the sugar content indicated the presence of glycoside in the extract.

Glycoside $\xrightarrow{HOH}$ Aglycon (genin) +Glycon (sugar)

6. Tests for Cardiac Glycosides[10]

- **Keller Killiani test**
- Sample was dissolved in 2 ml chloroform. H_2SO_4 was added to form a layer and the colour at interphase is recorded. Brown ring at interphase is characteristic of deoxy sugars in cardenolides.

- In 2 ml extract, GAA and 1drop of 5% of $Fecl_3$ and conc H_2SO_4 were added. Reddish brown colour appears at the junction of two liquid layers, and upper layer appears bluish green, confirming the presence of glycosides.

7. Test for Saponin Glycosides[9]

- **Foam test:** The drug sample or dry powder was shaken vigorously with water. Persistent foam was observed.

- **Hemolytic test:** drug sample or dry powder was added to one drop of blood placed on glass slide. Hemolytic zone was observed.

***8. Tests for Flavonoids*[9]**

- **Shinoda test:** Sample extract was treated with 5ml of 95% ethanol; few drops of conc. Hydrochloric acid and 0.5g of magnesium turnings were also added. Pink colour was observed. Addition of increasing amount of sodium hydroxide to the residue shown yellow coloration, which decolorizes after addition of acid indicates the presence of flavonoids.

***9. Tests for Tannins*[9]**

The sample was treated with 10% lead acetate solution; appearance of white precipitate indicated the presence of tannins.

When the extract was treated with aqueous bromine solution, appearance of white precipitate indicated the presence of tannins.

***10. Tests for Sterols*[9]**

The sample was treated with 5% potassium hydroxide solution appearance of pink colour indicates the presence of sterols.

Thin layer chromatography

TLC plates were prepared by using Silica Gel-GF 254 as adsorbent. 10gm silica gel-G was mixed with 20ml of distilled water (1:2) to make slurry. The slurry was immediately poured into the plates. Plates were then allowed to dry for two hours and the layer was fixed by drying at 100^0C for one and half hrs. Using a micropipette, about 10µl of extracts were loaded gradually over the plate and air dried. Then the plates were developed in different solvent systems such as chloroform: acetone: diethyl amine (5:3:2); dioxane: ammonia 24% (7:3); benzene: ethyl acetate (75:25); chloroform: methanol (7:3); toluene: dioxin: acetic acid (78:14:5); and chloroform: acetone: formic acid (64:12.3:3.2). The different solvent systems showed different R_f values for the same plant extract. The chromatograms were observed under visible light and were photographed.

The R_f values were obtained by using the following formula.

$$R_f = \frac{\text{Distance travelled by the solute (cm)}}{\text{Distance travelled by the solvent (cm)}}$$

RESULTS

Standardization: The whole plant powder has shown various values for several parameters. For loss on drying, it was dried at 103°C for 4 hours in an oven and then cooled, loss on drying was found to be 6.34 ± 0.12 and the total ash value was 4.54 ± 0.03. The result for water soluble ash, acid insoluble ash and sulfated ash was found to be 1.86 ± 0.07, 2.43 ± 0.04 and 1.56 ± 0.06, respectively. The pH of different formulations in 1% w/v (1g: 100ml) and 10% w/v (10g: 100ml) of water soluble portions of whole plant powder of *Mucuna pruriens* were found to be 5.11 ± 0.05 and 7.92 ± 0.05 respectively. The extractive values for both water and ethanol were found to be 18.65 ± 1.21and 11.52 ± 0.32 respectively. The results were given in the table 1.

Table. 1: Physicochemical parameters of *Mucuna pruriens.*

Parameters	Values
Loss on drying	6.34 ± 0.12
Total ash value	4.54 ± 0.03
Water soluble ash	1.86 ± 0.07
Acid insoluble ash	2.43 ± 0.04
Sulfated ash value	1.56 ± 0.06
pH of 1% w/v formulation solution	5.11 ± 0.05
pH of 10% w/v formulation solution	7.92 ± 0.05
Water soluble (hot) extractive value	18.65 ± 1.21
Ethanol soluble (hot) extractive value	11.52 ± 0.32

Extraction: The percentage yields of different extracts of *Mucuna pruriens* were found to be 4.9%, 4.5% and 5.5% for chloroform, ethyl acetate and methanol, respectively (Table 2).

Table. 2: Data showing the extractive values of *Mucuna pruriens.*

Plant name	Part used	% Yields of extracts (%w/w)		
		Chloroform	Ethyl acetate	Methanol
Mucuna pruriens	Whole plant	4.9%	4.5%	5.5%

Phytochemical screening

Preliminary phytochemical screening of *Mucuna pruriens* was carried out with three different extracts and the data was represented in Table 3. The preliminary phytochemical studies revealed the presence of alkaloids, steroids, glycosides and flavonoids in chloroform extract. Some phytoconstituents were observed in ethyl acetate extract viz., steroids, glycosides and flavonoids. Further, some phytoconstituents like alkaloids, proteins, amino acids, steroids, glycosides, flavonoids and tannins were shown in methanol extract.

Table. 3: Phytochemical constituents of *Mucuna pruriens* plant extracts.

S. No	Chemical test	Observation	Inference		
			Chloroform	Ethyl acetate	Methanol
1	**For alkaloids** Dragendorff's test Mayer's test	 Reddish brown precipitate White precipitate	++	--	++
2	**For Proteins** Biuret test(General) Millon's test Xanthoprotein test	 Violet/Pink color Red color precipitate Orange color	--	--	++
3	**For Amino acids** Ninhydrin test	 Purple/Bluish color	--	--	++
4	**For Steroids** Salkowski reaction Lieberman-Burchard reaction Liebermann's reaction	 Yellow Fluorescence Green color Blue color	++	++	++
5	**For Glycosides** Cardiac glycosides (Keller-Killiani) Test	 ---	+ +	++	++
6	**For Flavonoids** Shinoda test	 Pink color	++	++	++
7	**For Tannins** Test 1 Test 2	 White Precipitate White Precipitate	--	--	++

Thin layer chromatography

Chloroform extract: The thin layer chromatography analysis of *Mucuna pruriens* chloroform extract showed the presence of steroids with R_f values of 0.56 & 0.44 in benzene: ethyl acetate (80:20), chloroform: methanol (6:4) solvent systems correspondingly. Iodine vapours & UV-light were applied for the detection of steroids. Appearance of yellow zone and intense fluorescence indicated the presence of steroids in chloroform extract.

Ethyl acetate extract

The thin layer chromatography analysis of ethyl acetate extract of *Mucuna pruriens* showed the presence of flavonoids with Rf values of 0.55 & 0.45 in toluene: dioxin: acetic acid (78:14:5) chloroform: acetone: formic acid (64:12:3) solvent systems correspondingly. Iodine vapours & Natural products-poly ethylene glycol reagent (NP/PEG) were applied for the detection of flavonoids. Appearance of orange yellow zone and intense fluorescence indicated the presence of flavonoids in ethyl acetate extract.

Methanol extract

It was observed that thin layer chromatography analysis of *Mucuna pruriens* plant showed the presence of alkaloids in methanol extract. It was observed that the thin layer chromatography analysis of *Mucuna pruriens* methanol extract showed the presence of alkaloids with R_f values of 0.26 & 0.64 in chloroform: acetone: diethyl amine (5:3:2) & dioxane: ammonia 25% (7:3) solvent systems respectively. Marquis reagent & Ninhydrin were applied for the detection of alkaloids. Appearance of violet fluorescence and gray colour indicated the presence of alkaloids in methanol extract. R_f values of solutes separated from the various extracts of *Mucuna pruriens* was tabulated as Table No 4, 5 and 6.

Table. 4 Steroids: TLC studies for chloroform extract of *Mucuna pruriens.*

Solvent system for chloroform extract of *Mucuna pruriens*	Spraying reagent	Colour of spots	Rf value	Inference
Benzene: ethyl acetate (80:20) for chloroform extract of *Mucuna pruriens*	Iodine vapours	Yellow zone	0.56	Presence of Steroids
Chloroform: methanol(6:4) for chloroform extract of *Mucuna pruriens*	UV-light	Intense fluorescence	0.44	Presence of Steroids

Table. 5: Flavonoids: TLC studies for ethyl acetate extract of *Mucuna pruriens.*

Solvent system for ethyl acetate extract *Mucuna pruriens*	Spraying reagent	Colour of spots	Rf value	Inference
Toluene: dioxin: acetic acid (78:14:5) for ethyl acetate extract of *Mucuna pruriens*	Iodine vapour	Orange-yellow	0.55	Presence of Flavonoids
Chloroform: acetone: formic acid (64:12:3) for ethyl acetate extract of *Mucuna pruriens*	Natural products- poly ethylene glycol *reagent*	Intense fluorescence colour	0.45	Presence of Flavonoids

Table. 6: Alkaloids: TLC studies for methanol extract of *Mucuna pruriens.*

Solvent system for Methanol extract *Mucuna pruriens*	Spraying reagent	Colour of spots	Rf value	Inference
Chloroform: acetone: diethyl amine (5:3:2) for methanol extract of *Mucuna pruriens*	Marquis reagent	Violet fluorescence	0.26	Presence of Alkaloids
Dioxane: ammonia 25% (7:3) for methanol extract of *Mucuna pruriens*	Ninhydrin	Grey colour	0.64	Presence of Alkaloids

DISUSSION

Standardization is useful for defining crude drugs & it gives an idea about the nature of the chemical constituents present. Standardization can play an important role in the evaluation of herbal drugs and can also become an important quality control method as well as for proper stability testing of the product. Standardization is an essential measurement for ensuring the quality control of the herbal drugs.[11] India can emerge as the major country and play the lead role in the production of standardized, therapeutically effective ayurvedic formulation. India needs to explore the medicinally important plants. Standardization of herbal formulation is essential in order to assess the quality of drugs, based on the concentration of their active principles.[11] The traditional approach towards standardization is insufficient for current herbal market and hence there is need for more advanced techniques for standardization.

Phytoconstituents play an important role in the field of new drugs research and development because of their low toxicity, easy availability and cost effectiveness. Flavonoids are important group of polyphenols widely distributed among the plant flora. Structurally, they are made of more than one benzene ring in its structure (a range of C15 aromatic compounds) and numerous reports support their use as antioxidants or free radical scavengers. Phenolic compounds are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens. They have been the subject of a great number of chemical, biological, agricultural, and medical studies.[12] Flavonoids are important group of polyphenols widely distributed among the plant flora.[12] Many alkaloids, though poisons, have physiological effects that render them valuable as medicines. For example, curarine, found in the deadly extract curare, is a powerful muscle relaxant; atropine is used to dilate the pupils of the eyes; and physostigmine is a specific for certain muscular diseases[12] Tannin is an astringent, plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids.[12] The presence of various phytoconstituents in the plant *Mucuna pruriens* may be responsible for the different pharmacological activities of the plant.

Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. A large number of solvent systems were tried to achieve a good resolution. TLC profiling of all the extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals gave different R_f values in different solvent system. This variation in R_f values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the R_f values of compounds in different solvent system. Different R_f values of the compound also reflect an idea about their polarity. As seen in table (4,5 and 6) all the extracts were subjected to thin layer chromatography by using different solvent systems. The TLC profiling of all the extracts in different solvent systems confirms the presence of diverse potent bio molecules in these plants. TLC analysis Provide an idea about the polarity of various chemical constituents, in a way such that compound showing high R_f value in less polar solvent system have low polarity and with less R_f value have high polarity. These potent biomolecules can be further used for development of different drug in future.

CONCLUSION

The present work was taken up in the view to completely standardize the herb in accordance to parameters of World Health Organization (WHO) Guidelines and standard laboratory procedures. Extraction, standardization and phytochemical screening were done to evaluate the qualitative and quantitative parameters of *Mucuna pruriens.* The study of whole plant of *Mucuna pruriens* was thoroughly investigated for their organoleptic characters; physicochemical characters and major active constituents to analyze their superiority, protection and standardization for their safe use. The values of each parameter were found to be within the prescribed limits as per WHO. The phytochemical and TLC studies have shown that the plant extracts of *Mucuna pruriens* possess flavonoids, terpenoids and steroids were present in chloroform, ethyl acetate and methanol extracts. The results of the study also provide an evidence to conclude that the plant *Mucuna pruriens* plant parts can be used to treat various diseases like cancer and heart related diseases etc. The generated information of the present study will provide data which is helpful in the correct identification and authentication of this medicinal plant and may help in preventing its adulteration.

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