

**ANTIBACTERIAL ACTIVITY, PHYTOCHEMICAL ANALYSIS OF
METHANOLIC EXTRACT OF *Mucunapruriens***

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ABSTRACT

Mucuna pruriens is known as velvet Bean. All parts of *Mucuna pruriens* has been known to possess valuable medicinal properties. The purpose of this study was investigating experimentally the possible antibacterial activity of methanolic extract of *Mucuna pruriens* and phytochemical analysis of seed of *Mucuna pruriens*. Antibacterial activity of methanolic extract of *Mucuna pruriens* was evaluated at two different concentrations by diffusion method. The methanolic extract of *Mucuna pruriens* shows antibacterial activity at various levels in the *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* the bacterial *B. cereus* was found to be more active and *E.coli*, *S.aureus* was found to be less active in inhibition zone. Results exhibited that *Mucuna pruriens* contain good antibacterial action. Preliminary phytochemical analysis of *Mucuna pruriens* showed presence of flavonoids, phenol, tannin, saponin. Which may be active compounds. The results justify the *Mucuna pruriens* is medicinally important plants.

Keywords:-*Mucuna pruriens*, Methanolic extract, Antibacterial property, Phytochemical activity.

INTRODUCTION

Medicinal plants contains substances that can be used for therapeutic purpose or precursors for the synthesis of useful drugs¹. Medicinal plants were used by people of ancient cultures without knowledge of their active ingredients. The common practice of taking crude extract orally is laden with hazards as the extracts may contain some toxic constituents. There is an ever increasing need to limit toxic clinical drugs². Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines³. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health care since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play a vital role in modern drug development in the pharmaceutical industry⁴. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extract on bacteria have been studied by a very large number of researches in different parts of the world⁵. Much work has been done on ethnomedicinal plants in India⁶. Interest in a large number of traditional natural products has increased⁷. It has been suggested that aqueous and ethanolic extract from plants used in allopathic medicine are potential sources of antiviral, anti tumoral and antimicrobial agents. The selection of crude plant extracts for screening programmes has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products. The present results of our plant extract studies show that such extracts can be used by communities for curative purpose⁸⁻¹¹. *Mucunapruriens* also known as velvet bean, all parts of *Mucunapruriens* has been known to possess valuable medicinal properties. *Mucunapruriens* commonly known as cowhage or velvet bean or Athmagupthain India. The bean contains a high protein content but remains a minor food crop due to the presence of anti-nutrient compounds mainly 3,4-dihydroxy-L-phenylalanine (L-Dopa). It is rich in protein (23-35%) and its digestibility is comparable to that of other pulses, like soybean, rice bean and lima bean¹². *Mucunapruriens* is one of the many “under-utilized” tropical legumes that are widely used as a cover crop¹³. It is a vigorous, perennial, herbaceous climbing vine that has the capacity to grow up to 6m in length. The leaves are trifoliate with white or dark

purple flowers that hang in long clusters. The pods are sigmoid and the seeds aovoid, having 4-6 seeds per pod. The seeds vary in colour from black, white to mottle and the pods which are thick and leathery are covered with reddish orange long stiff hairs that are readily dislodged and can cause intense irritation to the skin due to the presence of a chemical known asMucunain¹⁴⁻¹⁶. *Mucunapruriens* is widely utilized by both humans and livestock for various purpose. Seeds of*Mucunapruriens* are also used as soup thickeners¹⁷.Velvet beans are used as a source of protein in diet of fish, poultry and cattle¹⁸. Pharmacologically, *Mucuna pruriens* seeds have been used as analgesics, anti-inflammatory, diuretics, anthelmintic, CNS stimulant, cough suppressant, antihypertensive etc.¹⁹*Mucuna puriens* contains an anti-nutritional factor L-DOPA which is used for the treatment of Parkinson's disease²⁰. *Mucunapruriens* has been reported to possess anti-diabetic, anti-neoplastic, anti-microbial, aphrodisiac, and learning and memory enhancing properties²⁰. Pro-male fertility properties of MP are supported by few studies including one of our studies on human subjects²¹⁻²⁷. The exact mechanism of its action remains elusive, but possibly it is the result of its anti-oxidant, adaptogenic and general nutritional properties²³⁻²⁵.

MATERIALS AND METHODS

All chemicals, media and reagents were used are AR grade.

Collection of plant Material

Fresh seed of *Mucunapruriens* were obtained from the plant grown in Ghulewadi areas,Sangamner, Dist-Ahmednagar, Maharashtra,India in September and seeds were cleaned and dried in shade at room temperature & finely pulverized in the Department of Drug Chemistry, S.M.B.S.T.College, Sangamner.



Kingdom: - Plantae
Order:- Fabales
Family:- Fabaceae
Subfamily:- Faboideae
Tribe:- Phaseoleae
Genus:- Mucuna
Species:- *M.pruriens*



Preparation of Methanolic Extract

Dry seed powder of *Mucuna pruriens* was continuously refluxed with methanol at 40°C-50°C for 72 hours using soxhlet apparatus. The solvent extract was then stored in air tight container at 4°C till further use.

Qualitative analysis of Phytochemicals

The analysis of phytochemicals from the solvent free extract of *Mucunapruriens* seed was individually carried out using various qualitative tests for alkaloids, carbohydrates, glycosides, flavonoids, steroids, terpenoids, saponin, tannin, protein, volatile oils & essential oils²⁸.Table 1.

Extraction of Phytochemicals

The individual phytochemicals was extracted in the appropriate solvent and stored in air tight container at 4⁰C till further use²⁸.

Test for Alkaloids

The small portion extract were stored separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal agents, such as Mayer's reagent (cream precipitate) dragendorff's reagent (orange brown precipitate).

Test for Carbohydrates and Glycosides

Small quantity of extract was dissolved separately in 5 ml of distilled water and filtered. The filtrate may be subjected toolisch's test to defect the absence of carbohydrates. Another small portion of extract was hydrolysed with dilute hydrochloric acid for few hours in water bath and was subjected to Liebermann-Burchard's test to detect absence of different glycosides (pink to red colour indicates presence of glycosides).

Test for Flavonoids

5 ml of dilute ammonia solution was added to a portion of aqueous filtrate of plant extract followed by addition of concentrated H₂SO₄. A yellow coloration absorbed in extract indicated presence of flavonoids.

Test of Steroids

2 ml acetic anhydride was added to 0.5 g ethanolic extract with 2 ml H₂SO₄. The colour changed from violet to blue it indicate presence of steroid.

Test for Terpenoids (Salkowski test)

5 ml extract was mixed in 2 ml of chloroform & 3 ml concH₂SO₄ was added carefully to form a layer. A reddish brown colorationof the interface was formed indicated presence of terpenoids.

Test for Saponin

1 ml extract and 1 ml alcohol diluted with 20 ml distilled water and shake well about 15 minutes. 1 cm layer of foam indicated presence of saponin.

Test for Tannin

Extract is treated with vanillin hydrochloric acid reagent pinkish red colour is formed it indicate the formation of phloroglucinol.

Test for Protein

Mellon's reaction: Million's reagent (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually yields a white precipitation on addition to a protein solution which turns red on heating.

Test for Volatile oil or Essential oil

Place a thick section of drug on glass slide. Add a drop of sudan red 3rd reagent and after two minute wash with 50% alcohol mount in glycerin.

Antibacterial Activity

Antibacterial activity²⁹⁻³⁰ of water extracted of *Mucunapruriens* was analyzed **Table 2**. Gram positive bacteria *Staphylococcus aureus*, *Bacillus cereus* & gram negative bacteria *Escherichia coli*, were used. Inoculum size was adjusted to 1 to 2×10^7 CFU (colony forming unit) / ml by serial dilution with sterilized nutrient broth media. Nutrient agar (pH 7.2-7.4) was used for routine susceptibility testing of non-fastidious bacteria. Stock solution of 10000 g / ml was prepared in 20% v/v water in DMSO. Using the stock solution 5000 μ g, 4000 μ g, 3000 μ g & 2000 μ g solution were prepared from which 150 ml solution was taken for assay. Ciprofloxacin was used as a standard 20 % v/v WFI in DMSO was used as a control. Antibacterial assay was carried out by agar well diffusion method³¹⁻³³ after 18 to 24 hrs of incubation.

Table 1. Phytochemical analysis of water extract of *Mucunapruriens* seeds

Sr.No.	Phytochemicals	Presence/ Absence	Sr.No.	Phytochemicals	Presence/ Absence
1	Alkaloids	+	7	Triterpenoids	+
2	Carbohydrates	++	8	Saponin	+
3	Glycosides	-	9	Tannin	++
4	Flavonoids	++	10	Protein	++
5	Steroids	+	11	Volatile oil	-
6	Amino acids	+	12	Phenol	+

Table 2. Zone of inhibition of different concentration of water extract of *Mucunapruriens* by the different methods

Sr. No	Bacteria	Reference substance	Inhibition Zone		
			100	150	200
1	<i>B.cereus</i>	39.67 + 0.81	12.70 + 0.24	17.22 + 0.53	26.47 + 1.98
2	<i>E.coli</i>	36.60 + 0.73	10.68 + 0.11	05.88 + 0.20	02.68 + 0.17
3	<i>S.aureus</i>	37.10 + 0.96	08.34 + 0.84	10.53 + 0.15	19.33 + 0.74
4	<i>K.pneumoniae</i>	36.94 + 0.77	09 05 + 0.43	11.67 + 0.87	17.11 + 1.08

Extractive values

The methanolic extract of *Mucunapruriens* used for extractive values, ash values, PH, refractive index and separation of total extractive into acids and neutrals Table 3

Table 3. Extractive values of *Mucuna pruriens*

Sr.No.	Parameters	Calculated values
1	Total ash	02.98 %
2	Total dissolved solids	29 .00%
3	Total acids	03.54 %
4	Total neutrals	07%
5	Refractive index	1.3452
6	pH	6.8

RESULTS AND DISCUSSION

The prediction of medicinal plants depends on the type of solvent used for extraction. Traditional medicinal plants use primarily methanol as a solvent. Hence in the present study we use methanol as a solvent for extraction of seed powder. Phytochemical screening suggested that water extract of *Mucunapruriens* contain various constituents³⁴. Phytochemical screening helps to reveal the chemical nature of the constituents of the *Mucunapruriens* extract. Table 1. Qualitative analysis showed the presence of the alkaloids, glycoside, steroids, tripenoids, protein. The flavonoids, protein, carbohydrates & tannin present in more quantity. Flavonoids were found in the extract and are potent water soluble antioxidants. The presence of tannin suggested the diuretic property of the plant. The wound healing property and antibacterial activity of the plant can be attributed to the presence of tannin. Antibacterial activity of methanolic extract was analyzed. The methanolic extract of *Mucuna pruriens* shows antibacterial activity at various levels. *B.cereus*, *E.coli*, *S.aureus*, *K.pneumoniae*. The bacteria *B.cereus* was found to be more active & *E.coli*, *S.aureus*, *K.pneumoniae* was found to be less active in inhibition zone Table 2. The methanolic extract of *Mucunapruriens* shows ash 02.98 %, total dissolved solids 29 .00%, total acids 03.54%, total neutrals 07%, refractive index 1.3452 & pH 6.8. Table 3.

CONCLUSION

The present study used as traditional medicine among the tribal and non-tribal in different localities of rural areas for the treatment of various diseases. For primary treatment most of the tribal and non tribals depends upon the medicinal plants. The phytochemical investigation of the certain medicinal plant will be helpful for evaluation of nutritive value and preparation of modern drugs and medicines. Phytochemical screening helps to reveal the chemical nature of the constituents of *Mucuna pruriens extract*. Phytochemical analysis of seed extract showed that, it contain alkaloids, carbohydrates, flavonoids, steroids, amino acids, triterpenoids, saponin, tannin, protein & phenol. The extract of *Mucunapruriens* shows antibacterial activity at various levels of 3 bacteria *B. cereus* was found to be more active, *E. coli*, *S. aureus* and *K. pneumonia* are less active. The observed antibacterial effects may be due to the presence of phytochemicals in the extract. The methanolic extract of *Mucunapruriens* shows ash, total dissolved solids, total neutrals & refractive index 1.3452 & pH 6.8.

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