IRJSE *Int. Res. J. of Science & Engineering,* 2020; Special Issue A7: 595-601 SJIF Impact Factor 6.68
ISSN: 2322-0015
RESEARCH ARTICLE **OPEN ACCESS**

Preliminary phytochemical analysis and antimicrobial activity of *Mucuna pruriens* (L.) DC. leaves.

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Manuscript Details
Available online on http://www.irjse.in
ISSN: 2322-0015

Cite this article as:
Khirade PD, Gond GS and Dudhe SS. Preliminary phytochemical analysis and antimicrobial activity of *Mucuna pruriens* (L.) DC. leaves, *Int. Res. Journal of Science & Engineering,* February, 2020, Special Issue A7 : 595-601.

ABSTRACT
Mucuna pruriens (L.) DC. belongs to family Fabaceae. It is recognized to have medicinally essential biologically active compounds. The phytochemical analysis is a valuable step in the detection of phytochemicals. It can also add valuable information which results in novel drug discovery. The present investigation was carried out to inspect phytochemical components and antimicrobial activity of *Mucuna pruriens* (L.) DC. In this study methanolic and chloroform extracts of leaves were utilized for phytochemical and antimicrobial screening using standard methods. The screening resulted in the detection of many important phytochemicals. Methanolic and chloroform extracts were not found active against strains of *S. aureus* and *E.coli.*

Keywords: *Mucuna pruriens*, phytochemical analysis, antimicrobial screening.

INTRODUCTION

Medicinal plants are of great importance to the health of human beings and communities due to the presence of bioactive chemical compounds in them i.e. phytochemicals. Phytochemicals provide medicinal attributes to the plants often referred to as secondary metabolites due to the fact that the plants manufacture them might also have little need for them. These chemical components produce a specific physiological effect on the human body. Solomon, *et al.* [1] suggested that all parts of the plant body can synthesize these biologically active compounds Edeoga *et al.*, [2] suggested that alkaloids, terpenoids, tannins, saponins and phenolic compounds are the most important bioactive groups of plants.

The present investigation aims to inspect and examine phytochemical components and antimicrobial activity of *Mucuna pruriens* (L.) DC. leaves. The plant species *Mucuna pruriens* (L.) DC. commonly-known as velvet been belonging to plant family Fabaceae is of particular interest due to its medicinal use and unique phytochemical contents.

METHODOLOGY

Plant Material:
For present phytochemical and antimicrobial activity investigation leaves of a plant species *Mucuna pruriens* (L.) DC. (Fig. 1) having a variety of medicinally important phytochemicals is utilized.

Methodology:
To carry out phytochemical and antimicrobial activity investigation following methods were adopted.

i) Extensive exploration:
A survey was carried out to the different forest areas of Ballarpur, Chandrapur district. Maharashtra to find out the *Mucuna pruriens* (L.) DC.

ii) Collection of plant material:
Fresh plant of *Mucuna pruriens* (L.) DC. was collected for further study. Naturally growing plant species under study was photographed. For the identification purpose plant was photographed along with flowering twigs. Certain photographs of the flowers were also taken to make identification easier. During collection leaves along with stems were collected and brought to the laboratory for further study.

iii) Identification of collected plant species:
Identification, taxonomical description and authentification was carried out by referring to different floras including Flora of Maharashtra State: Dicotyledons Vol I and II, Singh et. al. [3-4], Flora of Maharashtra, [5], Flora of British India [6], Flora of Chandrapur and Gadchiroli district Ph. D. thesis, Nagpur University Nagpur [7] and Ethnobotanical studies of Chandrapur and Gadchiroli district Ph. D. thesis, Nagpur University Nagpur [8]. Leaves of freshly collected plant species were selected for further study.

iv) Processing of Plant Material:
Leaves were detached from the stems. Detached leaves were washed thoroughly with tap water to remove dust particles followed by distilled water in the laboratory. These leaves were allowed to shed dry for about two weeks. The dried leaves were ground and sieved. The powder obtained was stored in zip-lock pouches and tested for the presence of various phytochemicals.

v) Extraction:
Powdered plant material was subjected to successive solvent extraction. For the extraction, Soxhlet extractor [9] with methanol and chloroform for 24 hrs method was adopted. The obtained crude mixture was evaporated and stored in a closed container in the refrigerator. The condensed extracts were used for preliminary screening of phytochemical constituents.

vi) Antibacterial Activity:
For screening of antimicrobial activity, two bacterial cultures *Staphylococcus aureus* and *Escherichia coli* were selected for the present investigation.

a) Preparation of microbial inoculums:
The fresh microbial cultures were prepared and used during the research period. The Nutrient Broth (NB) was prepared and poured into several tubes. Then pure microbial cultures were collected from the institute and inoculated in the tubes by using inoculation needles or loops. After these tubes were incubated (37^0C for 24-28 hrs for bacteria). After incubation, the cultures were used for the experiments.

b) Preparation of nutrient agar medium:
1000ml of Nutrient agar medium is prepared; pH was adjusted to 6.8. The medium is sterilized by using autoclave at 121 ^{0}C for 15 Ibs pressure for 15 minutes and allowed to cool.

c) Screening for antibacterial activity (agar well diffusion method):
The antibacterial activities of the plants were tested against the selected bacterial cultures. The 20 ml of sterilized Nutrient agar medium was poured into each sterile petriplates and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cotton swab. Then a

well of 6 mm are made in the medium by using a sterile cork borer, 150 µl of extracts were transferred into separate wells. After these plates were incubated at 37 ^{0}C for 24-28 hours. After the incubation period, the results were observed and measure the diameter of the inhibition zone around each well.

d) Antibiotic sensitivity test on bacteria (positive control):

The antibiotic sensitivity test was carried out using standard antibiotics (kanamycin, methicillin and ampicillin) and analyzed by the method of Bauer *et al.*, [10]. The sterilized nutrient agar medium was poured into each sterile petriplates and allowed to solidify. By using sterile cotton swabs, a fresh bacterial culture with known population count was spread over the plates by following the spread plate technique. Then the selected standard antibiotic disc was placed on the bacterial plates. Then the plates were incubated for 24 hours at 37^{0}C. After the incubation period, the results were observed and the diameter of the inhibition zone was measured around the isolates.

RESULTS AND DISCUSSION

The extract subjected to phytochemical screening showed the following results-

Results of Phytochemical screening

Methanolic Extract:

The extract showed positive tests indicating their presence for Phenols, Terpenoids, Reducing sugars, Alkaloids and Anthraquinone. Tests for Flavonoids, Tannin, Saponins and Cardiac glycosides of the extract were negative showing their absence in the extract (Fig. no. 2 and Table I.)

Chloroform Extract:

The extract showed positive tests indicating their presence for Phenols, Terpenoids, Tannins and Saponins. Tests for Reducing sugars, Alkaloids, Flavonoids, Cardiac glycoside and Anthraquinones of the extract were negative showing their absence in the extract (Fig. no. 3 and Table-II.)

Table I: Phytochemical constituents of *Mucuna pruriens* (L.) DC. leaves methanolic extract.

Phytochemical	Test	Inference*
Phenol	Ferric chloride test	+
Terpenoids	Chloroform test	+
Reducing sugar	Fehling's test	+
Alkaloid	Mayers test	+
Flavonoids	With aq:NaOH solution	-
Tannins	Ferric chloride test	-
Saponins	Foam test	-
Cardiac glycoside	Glacial acetic acid test	-
Anthraquinones	Conc. Sulfuric acid test	+

**+ (Presence) and – (Absence)*

Table II. Phytochemical constituents of *Mucuna pruriens* (L.) DC. leaves chloroform extract.

Phytochemical	Test	Inference*
Phenol	Ferric chloride test	+
Terpenoids	Chloroform test	+
Reducing sugar	Fehling's test	-
Alkaloid	Mayers test	-
Flavonoids	With aq: NaOH solution	-
Tannins	Ferric chloride test	+
Saponins	Foam test	+
Cardiac glycoside	Glacial acetic acid test	-
Anthraquinones	Conc. Sulfuric acid test	-

***+ (Presence) and – (Absence)**

Table III. Antimicrobial activity at different concentrations of methanolic extract of *Mucuna pruriens* (L.) DC. leaves.

Plant species	**Concentration (mg/ml)**	**Zone of inhibition of bacteria (mm)**	
		E. coli	***S. aureus***
Mucuna pruriens (L.) DC.	100 mg/ ml	No inhibition	No inhibition
	50 mg/ ml	No inhibition	No inhibition

Table IV. Antimicrobial activity at different concentrations of chloroform extract of *Mucuna pruriens* (L.) DC. leaves.

Plant species	**Concentration (mg/ml)**	**Zone of inhibition of bacteria (mm)**	
		E. coli	***S. aureus***
Mucuna pruriens (L.) DC.	100 mg/ ml	No inhibition	No inhibition
	50 mg/ ml	No inhibition	No inhibition

Fig. 1. *Mucuna pruriens* (L.) DC.

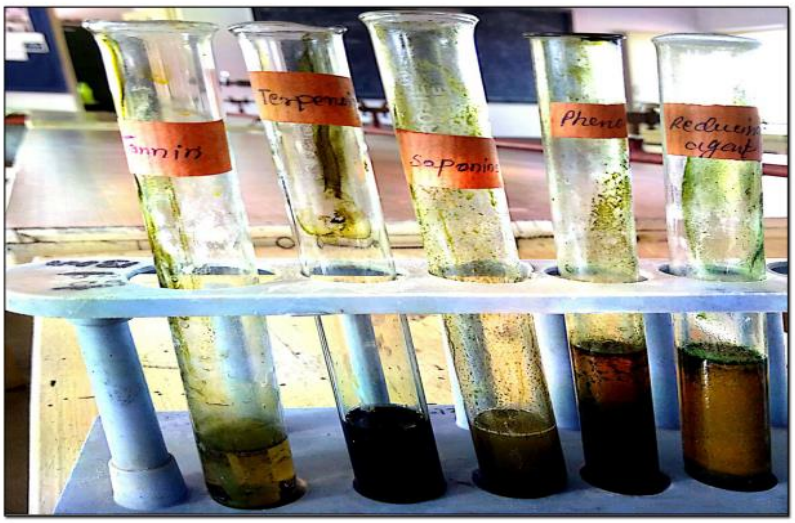

Fig.2: Phytochemical constituents test of *Mucuna pruriens* (L.) DC. leaves methanolic extract.

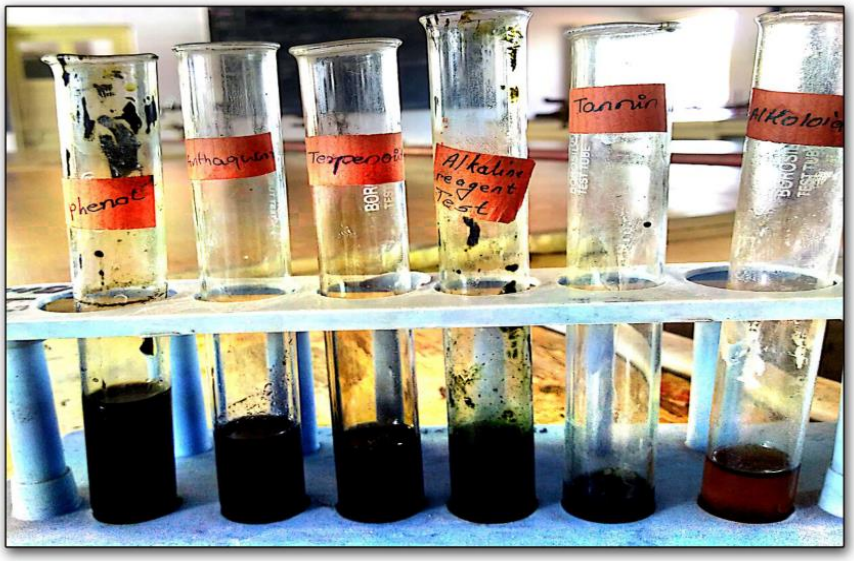

Fig.3: Phytochemical constituents test of *Mucuna pruriens* (L.) DC. leaves chloroform extract.

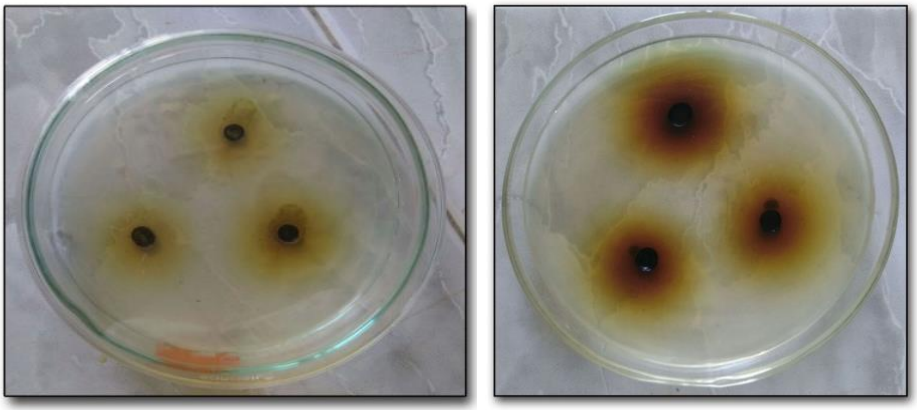

Fig.4: Antimicrobial activity at different concentrations of methanolic extract of *Mucuna pruriens* (L.) DC. leaves against *Escherichia coli*.

Fig.5: Antimicrobial activity at different concentrations of methanolic extract of *Mucuna pruriens* (L.) DC. leaves against *Staphylococcus aureus.*

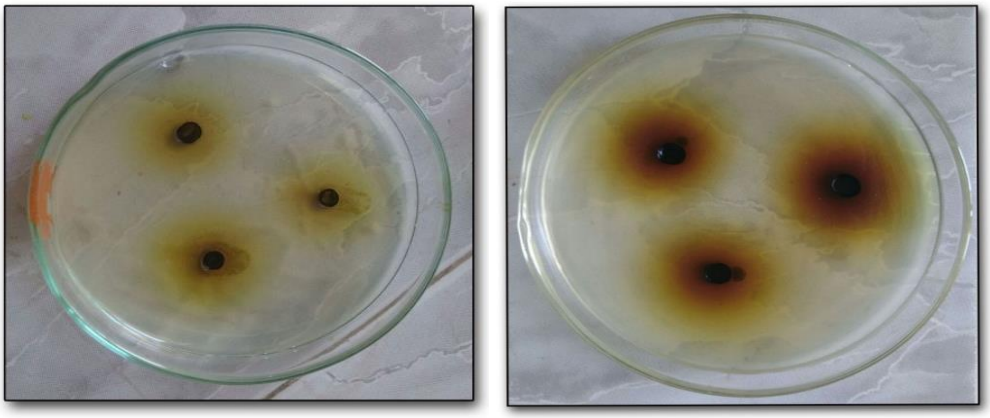

Fig.6: Antimicrobial activity at different concentrations of chloroform extract of *Mucuna pruriens* (L.) DC. leaves against *Escherichia coli*.

Fig.7: Antimicrobial activity at different concentrations of chloroform extract of *Mucuna pruriens* (L.) DC. leaves against *Staphylococcus aureus.*

Methanolic Extract:
Leaves methanolic extract have been found to have no *in vitro* antimicrobial properties at different concentrations. It was observed that the extract was not active against strains of *E.coli* and *S. aureus.* (Fig. No.4 and 5, Table-III)

Results of antimicrobial Activity
Antimicrobial activity of extracts was tested against *Escherichia coli.* and *Staphylococcus aureus.*

Chloroform Extract:
Leaves chloroform extract have been found to have no *in vitro* antimicrobial properties at different concentrations. It was observed that the extract was not active against strains of *E.coli* and *S. aureus* (Fig. No.6 and 7, Table-IV)

The phytochemical analysis and antimicrobial study of *Mucuna pruriens* (L.) DC. indicates the presence of medicinally important phytochemicals. The extract of leaves was not active against the microorganisms used.
Plants produce secondary metabolites (phytochemicals) to serve for their defenses. These secondary metabolites have important medicinal properties. These phytochemicals can be used to overcome health problems, preparation of drugs, preparation of medicines,etc. Many workers reported the presence of medicinally important phytochemicals in *Mucuna pruriens (L.) DC.* Some of them mentioned their medicinal properties and uses.

The phytochemical analysis of *Mucuna pruriens* (L.) DC. reveals the presence of such important phytochemicals including Tannins, Saponnins, Reducing sugars, Alkaloids, Terpenoids, Flavonoids and Anthraquinone. Uma, *et al* (2015)[11] carry out phytochemical screening of *Mucuna pruriens* (L.) DC. reported the presence of alkaloids, tannins, phenols, flavonoids and saponins. Okere, *et al* 2014 [12]studied *proximate analysis of the Mucuna pruriens leaves, they also found the presence of* Alkaloids, Saponins, Saponins, Flavonoids, Cardiac glycosides, Tannins, Anthraquinones, Terpenoids and Steroids.

A study carried out by Verma *et al* (2014)[13] revealed that the plant *Mucuna pruriens* (L.) DC. exhibit a wide array of phytoconstituents like alkaloids, flavonoids, tannins and phenolic compounds that are responsible for varied potent physiological and pharmacological activities. In summary, the data present in this report reveals that plant species *Mucuna pruriens* (L.) DC. is very important medicinally.

Conflicts of interest: The authors stated that no conflicts of interest.

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