



Establishment of *in vitro* Shoot Induction and an Evaluation of Antioxidant and Phytochemical Properties of *Mucuna pruriens*

Neha Saini¹, Pallavi Upadhyay², Naitik Bhardwaj², Indra Rautela², Ashmita Bhatt¹, Nishima Sharma¹, Jyoti Barthwal¹, Prity Kumari¹ and Naveen Gaurav¹

¹Department of Biotechnology, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Dehradun-248001, Uttarakhand, India.

²Department of Biotechnology, School of Applied and life Sciences, Uttaranchal University, Dehradun-248001

*Corresponding author: naveensri17@gmail.com

ABSTRACT

Therapeutic plants have filled in as a steady wellspring of medicaments, which have incredible viability and interest in the treatment of different sicknesses. One of the plants that merit consideration is *Mucuna pruriens*. *M. pruriens* is a fundamental healing plant filling in the shrubs, supports, and dry deciduous woods all through India. It is utilized in conventional homeopathic medication arrangements in India for the treatment of male virility and neurological infections. It is recorded that *M. pruriens* includes L-3, 4-dihydroxy phenylalanine (L-DOPA) a synapse antecedent, utilized for the fix of Parkinson's infection. It has been likewise utilized as a customary food in certain nations. The metal and phytochemical examination showed that seeds of velvet bean or *M. pruriens* can be consumed securely, in light of the fact that their fixation was beneath the most extreme level required. Phytochemical investigation exhibited the occurrence of steroids, tannins, saponins and alkaloids in the methanol concentrate of the plant. However, the primary phenolic part of *M. pruriens* is L-DOPA. It disconnects might be of remedial worth with respect to a few pathologies however this examination additionally worried about the *in vitro* shoot acceptance of *M. pruriens* in light of the fact that the enormous interest of L-DOPA is mostly welcomed by the drug business by the extraction of the build from wild populaces has prompted its deficient accessibility in normal condition.

Keywords: Male virility, *M. pruriens*, L-Dopa.

INTRODUCTION

Mucuna pruriens Linn (Fabaceae), otherwise called cowhage organic product, kapikacho, or kevach is the most normally utilized ayurvedic drug. *M. pruriens* is an under-used vegetable species filled transcendently in Asia, Africa, and certain pieces of America (Vadivel and Janardhanan, 2000). Among the wild vegetables, the class *Mucuna* is far-reaching in hot, humid, and sub-tropical regions of the biosphere and is measured as an elective protein source. Primarily in India, this vegetable has been considered as a therapeutic plant filled in certain pieces of Madhya Pradesh, Uttar Pradesh, Andaman and the Nicobar Islands. It normally fills in the entire tropical

fields of India from the scope of the lower Himalayans (Saini et al., 2021). Inhabitants of these states utilize these vegetables as customary medication and for the plan of conventional food items. It develops best in regions where yearly daytime temperatures are inside the scope of 20 - 30°C and Annual downpour fall going 1,000-2,000 mm is great for the development of *M. pruriens*. It is an important therapeutic plant used to deal with a few illnesses like jungle fever, malignant growth, epilepsy, Parkinson's infection, looseness of the bowels, helminthiasis, ulcer, fruitlessness, snakebite, scorpion stings, and elephantiasis (Lampariello et al., 2011; Okafor et al., 2013; Oyeyemi et al., 2019). The plant fills in as a cover crop normally

developed as successful green fertilizer to renew crushed soil because of its capacity to gather supplements in different conditions (Sathiyarayanan and Arulmozhi, 2007; Lorenzetti et al., 1998). It shows allelopathy to tidy development and is productive in diminishing the roundworm populace in ranches (Lampariello et al., 2011; Pugalenti et al., 2005).

A few examinations have exposed that L-DOPA (L-3,4-dihydroxyphenylalanine), isoflavanones, lectin, and a few alkaloids confined in seeds of *M. pruriens* are liable meant for the huge bioactivity of its unrefined concentrates (Dendup et al., 2014; Lacerda et al., 2015). As of late, a few remedial methodologies have been embraced to make a sound living reasonably for all no matter what their status in the general public, since engineered items are costly as well as accompanied by unfavorable impacts (Atanasov et al., 2015; Jimoh et al., 2019). The difficulties presented by the expense and security of manufactured medications have raised the necessity to investigate more or less used plant species rumors meant for huge therapeutic importance (Jimoh et al., 2019; Jimoh et al., 2018; Olatunji et al., 2019). From past reports, various pieces of *Mucuna pruriens* have been shown to stand really great meant for assorted remedial resolutions (Oyeyemi et al., 2019; Sathiyarayanan and Arulmozhi, 2007).

To add to the rising interest for plant-based items as regular cures in the drug business as well as in families and creation of the accumulate from wild substances has prompted its insufficient accessibility in normal conditions, so *in vitro* plant, culture procedures including organ culture and callus culture (Pugalenti et al., 2005; Dobhal et al., 2013; Sharma et al., 2013; Rautela et al., 2018) might be a reasonable option for proliferation and preservation of plant and this study tries to survey *in vitro* shoot recovery of *M. pruriens* and afterward assess the phytochemicals and cell reinforcement exercises of the plant. It is our educated assessment that the result of this examination will help drug businesses and families that might need to investigate the herbal plant as a diet complement or meant for additional restorative determinations. The present study was concluded with the establishment of *in vitro* shoot induction and determined the analysis of antioxidant and phytochemical properties of *Mucuna pruriens*.

MATERIAL AND METHODS

Plant Material

The plant material was collected from the botanical garden of Shri Guru Ram Rai University, Dehradun, Uttarakhand.



Figure-1: Showing the mother plant *M. pruriens*.

Determination of Moisture Content:

Dampness not set in stone by setting gauged test of 5 gm of medication in broiler for 5 hours at 105°C, and mass of test remained determined at regular intervals until the heaviness of the example emerged to stand consistent, no variety of mass remained noted. This taster was permitted to unruffled or cool down at ambient temperature in a crucible apparatus designed intended for an hour prior to gauging (The Ayurveda Pharmacopeia of India, 2007).

Crude Extraction

Ethanol and Methanol of the usual scientific category were used for extraction. Soxhlet method for the extraction was used. 50 gm of powder dried plant material used for the extraction and 250 ml each of Methanol and ethanol as a solvent used and Soxhlet run for 8 to 10 cycles and the remains after filtration were collected together and rigorous to 2% of its unique volume through vanishing at condensed compression in a rotatory evaporating apparatus. The gluey concentrate acquired remained gauged and the rate of production determined. The pre-arranged extricates were then put away in hygienic jugs set aside at 4°C in a fridge till additional utilization. The same technique was followed for ethanol extraction (Gaurav, 2016).

$$\text{Percentage Yield or produce (\%)} = \frac{\text{Weight of plant extract}}{\text{Weight of dry Sample}} \times 100$$

Qualitative phytochemical analysis

This test was performed to decide the optional metabolites bunches contained in the velvet bean extricate (Gaurav, 2016; Sardjon et al., 2012).

Alkaloid test: Alkaloid test was done by adding not many drops of Mayer's reagent to the arrangement of 0.5 g of concentrate in 1 ml of chloroform. Development of white accelerates show the presence of alkaloids. Mayer's reagent was ready by dissolving of 1 gram KI in 20 ml of refined water, and 0.271 g HgCl₂ was added to an answer of KI.

Tannin test: Test was finished by weighing 0.1 gram

of concentrate broke down in 2 milliliters of purified water and afterward mixing a couple of droplets of 1% FeCl₃. The presence of dim blue tone demonstrates the presence of tannin (phenolic).

Saponin test: Saponin test was led by thawing 0.1 gram of concentrate in 3 milliliters of refined water, formerly, at that point, shaken energetically for 10 minutes. The rise of froth demonstrates the presence of saponin.

Steroid test: Steroids test was led by thawing 0.1 g of concentrate in 1 ml of distilled water, formerly, at that point, add up of 1 ml of CH₃COOH and 1 ml of concentrated H₂SO₄ to it. The rise of the blue or purple tone demonstrates the presence of steroids.

Flavonoids test: Flavonoid test was led by thawing 0.1 gram of concentrate in 3 milliliters of refined water and afterward add up of 0.1 g of Magnesium dust and 1 ml of concentrated HCl. The yellow tone shows the presence of flavonoids.

Determination of Heavy metals

The occurrence of heavy metals such as As, Ni, Co, Pb, Hg, Ag and Zn compounds remained attempted through various techniques (Sharma et al., 2017).

As: 10.0 milligram of debris was added up in an experimental tube and broke up although tenderly warming with 5 milliliters of somewhat acidic water and hypo phosphorus substance was added up, an earthy coloured accelerate was shaped.

Ni: Thawed 20 milligram of debris of the medication in around 0.5 milliliter of water, fermented through a couple of droplets of weaken HCL corrosive, and afterward adding up of drop by drop a weaken arrangement of NaOH. A blue hasten is framed which becomes green in colour on heating.

Co: Thawed 20 milligram of the slag of the medication in around 0.5 milliliters of distilled water, and fermented with a couple of drops of weaken HCL corrosive. Adding up of a couple of droplets of weaken arrangement of NaOH. A blue encourage is shaped which becomes pink on heating.

Pb: Thawed 0.1 gram of the constituent being inspected in 1millilitre of weaken acidic corrosive or utilize 1 milliliter of the endorsed arrangement. Adding up of 2 milliliters of K₂CrO₄ arrangement; yellow in colour accelerate insoluble in 2 milliliters of 10 molar NaOH was created.

Hg: Thawed 20-25 milligram of the slag of the medication in 1 milliliter of distilled water and adding up of KI arrangement. A red colour hasten is shaped that disintegrated in an overabundance of the chemical constituent.

Ag: Thawed 20-25 milligrams of slag of the medication in 2-3 milliliters of refined water and adding up of 0.2 milliliters of 7 molar HCL corrosive. A white curd type accelerate is shaped that is solvent in 3 milliliters of 6 molar alkali. Adding up of a couple of droplets of a 10% w/v fluid arrangement of KI a yellowish encourage is created.

Zn: Thawed 20-25 milligram of the slag in 2-3 milliliters of distilled water and adding up of 0.2 milliliters of 10 molar NaOH. A whitish encourage or precipitate is shaped which is disintegrated in 2 milliliters of 10 molar NaOH arrangement. Adding up of around 5 milliliters of 2 molar NH₄Cl along with 0.1 milliliters of Na₂S arrangement. A woolly, whitish accelerate is created.

Antioxidant studies

DPPH radical-scavenging activity of the extract

0.2 mm DPPH prepared by dissolving 7.8 mg DPPH solute in the 99.5 % ethanol and volume make up by 100 ml and it was kept in dark for about 2 hours until the absorbance stabilized. Another reagent used for the test is 0.1 Molar Tris buffer solution of HCL (pH-7.4) was prepared. Subsequently that 200 microlitre of an analytical taster solution and 800 µl of 0.1 molar Tris HCL were added into a test tube, 1 milliliter of DPPH solution adding up instantly, then solution was assorted with a solution mixer for 10 sec. Afterward, it remained kept at normal ambient temperature. After half an hour of the addition of DPPH and the optical density of the solution at 517 nm was measured and a mixture of ethanol, tris HCL and DPPH used as blank. Gallic acid used as standard (Anosike et al., 2019).

% scavenging activity of DPPH = (OD of control – OD of sample) / (OD of control) × 100

Estimation of vitamin E content of the extract

An amount of the example (2.5 milliliters) remained set in 2 experimental test tubes; 0.5 milliliter HNO₃ corrosive was adding up of to each and every tube and put for 3 min in bubbling water. Experimental tubes were chilled and permitted to remain for 15 min in obscurity. The capacity of arrangement in each Experimental tube's volume makes up to 5 milliliters with ethanol and optical density estimated at 470 nanometres. The centralization of vitamin E in the experimental arrangement was resolved utilizing an alignment bend of typical vitamin E fixation. A clear arrangement comprising 2.5 milliliters of refined water was placed in an experimental tube and 0.5 milliliter HNO₃ corrosive added up and set for 3 min in a bubbling water. The cylinder was chilled and set aside in obscurity. The standard was arranged utilizing a case of 1000 milligram vitamin E (Anosike et al., 2019).

Shoot Induction

For the shoot induction, the nodal explant of *M. pruriens* was surface sterilized by treating with a detergent (Tween 20, etc), and then with 0.1 % (w/v) of PVP and with a fungicide for 15 to 30 min then 0.1 % (w/v) mercuric chloride (HgCl₂) aqueous solution for 1 minute followed through systematic washing in disinfected distilled water. The formerly sterile explant was inoculated on MS media supplemented with 3% of sucrose and 8% of agar with different concentrations of cytokinin (50 mg-100 mg of BAP), and auxin (50- 100 mg of NAA and 100- 200 mg of 2,4-D). After that, the culture was nurtured in the culture incubation room at 25°C±1°C and with 55 % maximum comparative dampness (Saini et al., 2021; Gaurav et al., 2018; Pant et al., 2021).

RESULT AND DISCUSSION

Moisture and Ash Content

How much water Ash content in the material is related with the virtue and the presence of impurities in the simplicia. Assurance of debris content gives an outline of inward and outer mineral substance got from the underlying system to get great simplicial and extricates got from normal plants and pollutants during the interaction. The quantity of the greatest permissible debris content is related with virtue and pollution. Water and ash content contained in seeds of *M. pruriens* are shown in Table-1.

Table-1: Showing the percentage of Moisture and Ash content in *M. pruriens* seeds.

S.N.	Parameters	% In seeds of <i>M. pruriens</i>
1	Moisture content	9.70
2	Ash content	2.89

Extract of leaves of *M. pruriens*

Table-2 illustrates the produced percentage of the methanol and ethanol extract of the plant compared to the weight of the dried plant sample formerly extracted. The desiccated weight (50 grams) of the crushed sample gave 6.86% of ethanol extract and 7.9% of methanol extract.

Table-2: Produced percentage of extract of *M. pruriens* plant material.

S.No.	Solvents used for extraction	Weight of desiccated plant sample used (gram)	Weight of extract (gram)	Percentage of plant (%) produced	Colour of extract
1	Methanol	50	3.95	7.9	Dark Green
2	Ethanol	50	3.43	6.86	Dark Green

Phytochemical Availability in the Methanol extract of *M. pruriens*.

Table-3 illustrates the consequences of the phytochemical screening of the plant extract which uncovered the availability of a few significant bio-actives in mixtures like Flavonoids, steroids, tannins, saponins, and alkaloids.

Table-3: Bioavailability of phytochemicals in methanol extract of *M. Pruriens* leaves.

S.No.	Phytochemicals	Bioavailability
1	Flavonoids	Highly available
2	Alkaloids	Moderately available
3	Tannins	Moderately available
4	Saponins	Highly available
5	Steroids	Highly available

Qualitative determination of Heavy Metals:

Metal subjective tests were performed to decide the number of metal foreign substances that were contained in the example. Table-4 shows the results of qualitative tests including Lead (Pb), Silver (Ag), Tin (Sn), Mercury (Hg) and Arsenic (As), Nickel (Ni), Cobalt (Co), Zinc (Zn).

Table-4: Shows the heavy metal contaminant in the ash of seeds of *M. pruriens*.

S.No.	Heavy Metals	Result
1	As	Negative
2	Ni	Negative
3	Co	Negative
4	Pb	Negative
5	Hg	Negative
6	Ag	Negative
7	Zn	Negative

Antioxidant Activity:

Antioxidant Vitamins E Amount in the Methanol extract of *M. pruriens* leaves

Table-5 demonstrates the amount (milligram/100gram) of antioxidant vitamins E having in the extract of *M. pruriens*. The amount of vitamin E was 99.58 milligram/100gram.

Table-5: Showing the Vitamin E concentration.

Constituent	Amount (mg/100g)
Vitamin E	99.58

1,1 diphenyl-2-picrylhydrazil (DPPH) free radical scavenging activity

The activity of antioxidants in the methanol extract of *Mucuna pruriens* plant leaves utilizing DPPH measure had been displayed in Fig. 2. The different convergences of the concentrate displayed huge antioxidant action. The % rummaging action of the DPPH extremist through the

concentrate for the various groupings of 0.375, 1.2, 1.5, 3.14 and 3.58 milligram/milliliter were 57, 61, 72, 76, and 80 % respectively (Figure-2).

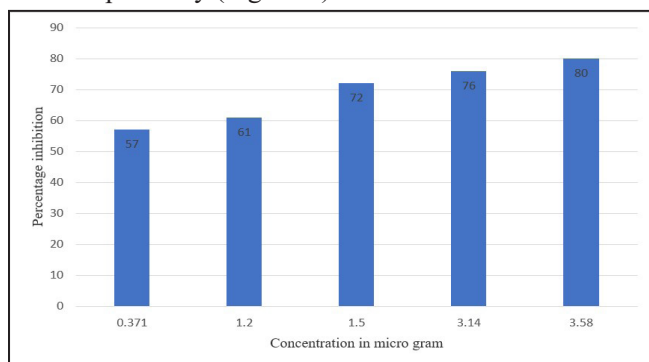


Figure-2: DPPH percentage inhibition and radical scavenging activity.

Shoot Regeneration:

Table-6 shows the result, after 2 weeks of incubation in a culture incubation room at 25°C±1°C and with 55 % maximum comparative humidity of culturing nodal explant getting a shoot regenerate in the MS media augmented with 3 % of sucrose and 8 % agar, and concentration and combination of growth regulators such as BAP, NAA and 2,4-D (Figure 3).

Table-6: Showing the no. of shoot regenerate of *M. pruriens* in the different concentrations of Auxin and Cytokinin

S.No.	Medium	Hormone taken (mg/l)	No. of explant	No. of shoots
1	MS	50 BAP+50 NAA+100 2,4-D	10	7
2	MS	50 BAP+100 NAA+100 2,4-D	10	3

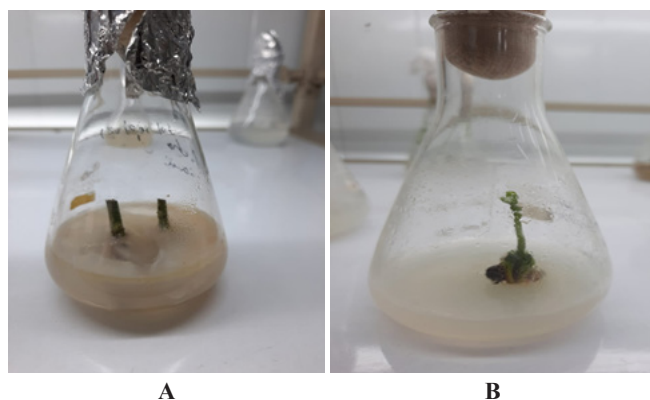


Figure-3: A- cultured nodal explant, B- The regenerated shoot of *M. pruriens*.

CONCLUSION

The current research demonstrated that *M. pruriens* could be used as an antioxidant, extract of plant leaves has shown more than 80 % antioxidant activity. The metal

examination showed that both seed and leaves were as yet ok for utilization, they are beneath the most extreme level. Phytochemical examination showed that both the samples contain an alkaloid, tannin, saponin, flavonoids and steroids. All plant parts of velvet beans such as leaf, stem, seeds and root have been portrayed to have remedial purposes and it has been considered in a few relations, for example, it has been shown antioxidant activity. The data shows that *in vitro* shoot recovery innovation could be an expense proficient method for high return creation of the best establishing material consistently, with next to no occasional imperatives. Albeit furthermore work is expected to take advantage of the all-out likelihood of the plant in the area of pharmacology.

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