Preliminary Phytochemical Study, *Invitro* Anti-Microbial and Anti-Oxidant Activity of Alcoholic Extract of *Mucuna Pruriens* Leaves

Ramya Kuber B*, Swapna B, Tejaswi M, Triveni M

Department of Pharmacognosy, Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India, 51072.

**ABSTRACT**

The antioxidant activity of alcoholic extract of leaves of *Mucuna pruriens* (MP) of family Fabaceae was studied using three *invitro* assays. The antioxidant activity of alcoholic extract of MP was evaluated by scavenging of DPPH, Lipid Peroxidation, Nitric oxide Scavenging activity. The concentration of alcoholic extract were (3-800 µg/ml). The MP showed maximum scavenging activity at concentration of 800 µg/ml. The percent inhibition of the scavenging of the DPPH was found to be 80%, Lipid Peroxidation was 86% and nitric oxide scavenging activity was found to be 69%. The antibacterial activity of the alcoholic extract of *Mucuna pruriens* leave was carried out and it was determined by the cup plate and disc plate methods against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis. Streptomycin was used as a positive control. The zone of inhibition of alcoholic extract of MP against various microorganisms was measured and compared with standard control, MP showed maximum activity at the concentration of 200 mg/kg against all the bacterial strains, Maximum antibacterial activity against Bacillus subtilis and Pseudomonas aeruginosa and exhibited moderate antibacterial activity against Staphylococcus aureus and Escherichia coli. on the basis of the results obtained in the present study, the antioxidant activity of MP and antimicrobial activity may be due to presence of the active principle present in MP leaves. These *invitro* assays indicate that this plant extract is a significant source of natural antioxidants, which might be helpful in preventing the progress of various oxidative stresses. The finding justify the therapeutic application of the plant in the indigenous system of medicine, augmenting its therapeutic value. Preliminary Phytochemical analysis showed the
presence of flavonoids, alkaloids, tannins, amino acids, proteins, glycosides, carbohydrates etc. Phytochemical analysis intended to serve as a major resource for information on analytical and instrumental methodology in the plant sciences.

Key words: Antioxidants, Mucuna pruriens, Free radicals, Antibacterial activity, Cup plate method, Disc plate method, Phytochemical.

INTRODUCTION
Antibacterial of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic microbial. An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic).

Disinfectants are antimicrobial substances used on non-living objects or outside the body \(^1\). However synthetic antimicrobial compounds having adverse effects like abdominal pain, diarrhea, cramping, headache, nausea, vomiting, abnormal bleeding, injury to red and white blood cell precursor, hepatitis, dermatitis, haemolysis, kidney damage, immunosupression. Free radicals may be defined as chemical species associated with an odd or unpaired electron. They are neutral, short lived, unstable and highly reactive to pair up the odd electron and finally achieve stable configuration. They are capable of attacking the healthy cells of the body, causing them to lose their structure and function \(^2\).

Naturally there is a dynamic balance between the amount of free radicals produced in the body and antioxidants to scavenge or quench them to protect the body against stress effects. The amount of antioxidant principles present under normal physiological conditions may be insufficient to neutralize free radicals. Therefore, it is obvious to enrich our diet with antioxidants to protect against harmful diseases. Hence, there has been an increased interest in the food industry and in preventive medicine in the development of "Natural antioxidants" from plant materials. Hence, plants with antioxidant properties are becoming more and more popular all over the world \(^3\).

Hence efforts are being made to search easily available alternative medicines with low cost, having low or free of side effect. There is sample literature on preliminary phytochemical surveys and the knowledge of the chemical constituents of plants is desirable to understand herbal drugs and 'their preparations. This study will be helpful to isolate
and characterize the chemical constituents present in those plant extracts.

*Mucuna pruriens* (Fabaceae) is a topical legume known as velvet bean or Cowitch and by other common names found in Africa, India and the Caribbean. The telugu name is Duradagondi. The plant is an annual, climbing shrub with long vines that can reach over 15 m in length. When the plant is young, it is almost completely covered with fuzzy hairs, but when older, it is almost completely free of hairs. The leaves are tripinnate, ovate, reverse ovate, rhombus-shaped or widely ovate. The sides of the leaves are often heavily grooved and the tips are pointy. In young *M. pruriens* plant, both sides of the leaves have hairs. The stems of the leaflets are two to three millimeters long. Additional adjacent leaves are present and are about 5 mm long. The flower heads take the form of axially arrayed panicles. The plant is infamous for its extreme itchiness produced on contact, particularly with the young foliage and the seed pods. It has value in agricultural and horticultural use and has a range of medicinal properties [3]. The seeds of velvet beans are high in protein, carbohydrates, lipids, fiber, minerals, alkaloids, alkyamines, arachidic acid, behenic acid, saponins and sterols. The seeds of all *Mucuna* species contain a high concentration of L-dopa (7-10%). Pods, leaves and fruits contains serotonin and mucunain, which cause skin irritation and itch. cystine, dopamine, flavones, glutathione, 5-hydroxytryptamine, l-dopa [4]. The leaves contain about 0.5% L-DOPA, 0.006% dimethyltryptamine (DMT), 0.0025% 5-MeO-DMT and 0.003% DMT n-oxide [4]. Traditionally, *M. pruriens* has a sexual function improving effect in rats. It is used in Ayurvedic medicine [5]. It is used in the prophylactic treatment of snakebites [6] and has antidepressant, formulations of the seed powder have shown in the management and treatment of Parkinson's disease. Dried leaves of *M. pruriens* are sometimes smoked [4]. Traditionally, velvet bean has been used as a nerve tonic for nervous system disorders [7]. The hypoglycemic effect of the aqueous extract of the seeds of *Mucuna pruriens* was investigated in normal, glucose load conditions and streptozotocin (STZ)-induced diabetic rats. Investigated plasma proteome changes identify proteins responsible for survival of mice (Challenged mice). The antioxidant activity of *Mucuna pruriens* was demonstrated by scavenge DPPH, ABTS and ROS [8]. *Mucuna pruriens* significantly inhibited the lipids and deoxyribose sugar [9]. The D-chloro-inositol and its two galacto-derivatives were isolated in *Mucuna pruriens* seeds [10]. *MP* possesses significant higher antiparkinsonian activity compared with levodopa in the 6-hydroxydopamine (6-OHDA) lesioned rat model of Parkinson's disease. Four tetra hydro isoquinoline alkaloids isolated from *MP* seeds out of them, two are new whose structures have been elucidated by spectroscopic methods [11].
MATERIALS AND METHODS
Collection and extraction of plant material
Mucuna pruriens leaves was collected from Srinivasa Mangapuram, Talakona Region in Tirupati in the month of October and plant was identified by the Botanist Dr. Madhava Chetty, Department of Botany, SVU, Tirupathi. Alcoholic extract of the leaves of MP was prepared by cold maceration method. The collected leaves were washed and dried thoroughly and was powdered with the help of electric blender. 25 gm of shade dried powder was cold macerated and extracted successively with ethanol. The solvent extract was concentrated under reduced pressure. And obtained extract was grayish green in colour and percentage yield was 9.4% and it was stored in desiccator until used.

Growth and maintenance of test organisms for Anti microbial studies
Bacterial cultures of Escherichia coli; Pseudomonas auregenosa; Bacillus Subtilis; Staphylococcus aureus were obtained from culture collection centre; Department of microbiology. SriPadmavathi MahilaVishwavidhyalayam (SPMVV) were used for Antimicrobial test organisms. The bacteria was maintained on nutrient broth at 37°C.

Chemicals
Chemicals and solvents were of analytical grade were obtained from the store of IPT; SPMVV, Tirupati. DPPH, sodium nitro-prusside. sulphanilamide, potassium superoxide, O-phosphoric acid, napthyl, EDTA, KCl, FeSO₄, TBA, TCA, BHT, NBT, DMSO, NaOH. Distilled water, peptone, Agar-Agar, Meat extract, NaCl, Ethanol.

Invitro antioxidant studies
Preparation of rat brain homogenate
Randomly selected rats were fasted overnight. They were sacrificed by cervical dislocation, dissected and the whole brain was removed quickly. It was further processed to get 10% homogenate in 0.15 M KCL using a Teflon homogeniser. The homogenate was filtered to get a clear solution and used for further studies.

In vitro Lipid Peroxidation
The extent of lipid peroxidation in rat brain homogenate was measured in vitro in terms of formation of thiobarbituric acid reactive substances. Different concentrations of the plant extracts (3, 6, 12, 25, 50, 100, 200, 400, 800 µg/ml) were made up in alcohol. The alcoholic
extract was expressed in terms of dry weight (mg/ml) in alcohol. These samples were individually added to the brain homogenate (0.5ml). This mixture was incubated with 0.15M KCL (100 µl) and lipid peroxidation was initiated by adding 100 µl of 15mM FeSO₄ solution. The reaction mixture was incubated at 37°C for 30 min. One ml of TBA: TCA (1:1) was added to the above solution and then 1 ml Butylated hydroxy toluene (BHT). This final mixture was heated on a water bath for 20 min at 80°C and cooled, centrifuged and absorbance was read at 532 nm. Using a Spectrophotometer (Systronics, Model No. 106). The percentage inhibition of lipid peroxidation was calculated using the followed formula.

**DPPH radical scavenging activity**

DPPH scavenging activity was measured by the Spectrophotometric method. To an ethanolic solution of DPPH (200 µM), 0.05ml of test compound dissolved in ethanol was added at different concentrations (3-800 µg/ml). An equal volume of ethanol was added to the control. After 20 min the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition was calculated.

**Scavenging of nitric oxide radicals**

Nitric oxide (NO) is a free radical, excess production of NO is associated with several diseases. Sodium nitroprusside (5mM) in a standard phosphate buffer solution was incubated at 25°C for 5 hrs with different concentrations (3-800µg/ml) of the alcoholic extract in phosphate buffer (0.025 M, pH 7.4). Control was run without the test compounds but with the equivalent amount of the buffer in an identical manner. After 5 hrs, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess reagent (1% Sulphanilamide, 2%, O-Phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthyl ethylene diamine was read at 546 nm.

**Reducing Power Method**

This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. In this method antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples.
\[ Inhibition(\%) = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100 \]

**Preliminary Phytochemical analysis**

Preliminary Phytochemical analysis was carried out according to standard protocol [18].

**Preparation of stock solution**

One gram of the alcoholic extract of *Mucuna pruriens* leaves were dissolved in 100 ml of its own mother solvents to obtain a stock of concentration of 1% (w/v). The extracts thus obtained were subjected to preliminary phytochemical analysis.

**Anti-microbial activity**

**Assay of Antibiotic (Streptomycin) by the paper-disc plate method**

**Medium.** Bacto-streptomycin assay agar was prepared to provide a supply of a standard, uniform medium for the assay of streptomycin. The medium is prepared by dissolving 25.5 g per 1,000 ml in double distilled water. After sterilization the medium was stored at 2 to 4 °C until used.

**Organism**

A strain of Bacillus subtilis sensitive to streptomycin is employed as the test organism. A stock spore suspension is prepared by cultivation of the organisms on agar or in submerged culture. When microscopic examination reveals good sporulation, the cells are separated from the medium, suspended in sterile 0.05 M potassium phosphate buffer, pH 7.0 and the suspension pasteurized to kill the vegetative cells. A viable spore count is made by plating. The stock spore suspension is stored at 2 to 4 °C and used as needed.

**Preparation of plates**

To the Petri dishes (Pyrex dishes, 100 mm in diameter, selected for uniform flat bottoms 2) 20.0 ml of sterile assay agar was added. The agar is allowed to harden with the Petri dish tops tilted or removed. While the plates are still warm, 4.0 ml of seeded agar is added and distributed evenly over the medium depends on the concentration of viable spores. The amount to be used is best determined by actual test in the assay procedure, that quantity being selected which gives large, sharp zones. Usually a concentration 'of approximately 250, 000 spores per ml in the seeded agar gives the desired results. The spores are added to the liquefied agar at 60°C, and. the seeded medium is maintained at that temperature until all the
plates are poured. The plates are stored at 2 to 4 °C as soon as they have hardened and may be kept for several days with no effect on the assay. It is essential that the plates be prepared on an absolutely level table top.

Preparation of the sample

All samples are diluted on the basis of their estimated potency to fall on the standard curve. The initial dilution of liquid samples is made with an equal volume of 0.2 M potassium phosphate buffer, pH 7.9, and all subsequent dilutions with 0.1 M buffer. Solid samples are dissolved directly in 0.1 M buffer. Samples of submerged-culture beers are not clarified before dilution.

Setting up the assay

Filter paper discs' are placed, flat side down, on the agar plates. As each disc is placed, an 0.080-ml sample is immediately (within 5 seconds) pipette onto it. Since the disc rapidly absorbs moisture from the agar it is exceedingly important that the sample be applied in the shortest possible time. As the sample is being delivered the disc is gently pressed to the agar with the tip of the pipette. Four to six discs may be placed on one plate symmetrically arranged around the center. All discs should be equidistant and not less than 10 mm from the edge. A standard is included on each plate. Two to four replicates of each unknown are run on one or more plates.

Assay agar with 30°C incubation streptomycin

Plates containing the streptomycin standard are prepared daily, four to eight replicates of each of six dilutions of the standard being used. The concentration range of the standard depends on the other conditions of the procedure and is chosen to obtain a range of zone diameters of from 20 to 30 m. Under optimum conditions a concentration as low as 0.5 unite per ml (zone diameter 16 to 18 mm) can be determined. Not more than four to six plates are removed from the refrigerator at one time. These are handled as rapidly as possible and placed in the incubator as soon as they are completed (usual elapsed time, 15 minutes). They may be inverted during refrigeration and incubation.

Incubation of plates

The plates are incubated at 30 °C for a minimum of 15 hours. After this time no significant change in the size of the zone occurs up to 30 hours of incubation. At 37 °C the zone size is reduced, and on long incubation the organism tends to over grow the cleared area. However,
the zones develop more rapidly at 37 °C and the readings may be made after 4 to 6 hours of incubation thus affording a shorter assay time.

**Estimation of potency**

The diameters of the zones of inhibition are measured, to the closest one-quarter millimeter and replicates are averaged. A daily curve is prepared from the dilutions of the standard by plotting the zone diameters in mm against the concentration in units per ml. The potency of the unknown samples is determined from the standard.[19]

**The agar cup-plate method**

**Technique:** Use a "standard" organism. If Staphylococcus aureus is the test organism, growth of the bacteria for 18 to 24 hours in broth and standardize the suspension to one billion organisms per ml. Extract agar, pH 6.8. The final agar concentration should be 1.5 %. Seed the agar medium on the basis of 0.1 ml of the standard suspension of organisms for each 30 ml volume of medium. The temperature of the agar should be about 48°C. Pour approximately 30 ml of seeded agar medium into a standard 100 x 15 mm Petri dish bottom. Cover the Petri dish with a Coors porcelain lid glazed on the outside. After the agar solidifies, remove a disc of agar measuring 1.5 cm. in diameter. In some instances it is feasible and desirable to prepare three or more cups from a single plate. Prepare appropriate dilutions of the anti septic. Using a graduate 1ml. pipette, place 0.2 ml of a given dilution in each cup. The antiseptic should be added and the plates should be ready for incubation within one hour from the time the bacteria are added to the agar medium. Incubate test plates up right at 37 °C for 24 hours and then measure the width of the zone of inhibition of bacterial growth with a transparent ruler. The use of a hand lens in measuring the zones is an aid in obtaining accurate results.[20]

**RESULTS AND DISCUSSION**

Table. I Antibacterial Activity of alcoholic extract of *Mucuna pruriens* leaves

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zones of inhibition (mm)³</th>
<th>Streptomycin (1 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Mucuna pruriens</em> (mg/ml)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>160</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>45</td>
<td>15</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>38</td>
<td>14</td>
</tr>
<tr>
<td><em>Pseudomonas auregenosa</em></td>
<td>34</td>
<td>18</td>
</tr>
</tbody>
</table>

³The diameters of the zones of inhibition are measured, to the closest one-quarter millimeter and replicates are averaged. A daily curve is prepared from the dilutions of the standard by plotting the zone diameters in mm against the concentration in units per ml. The potency of the unknown samples is determined from the standard.[19]
Table. II Antioxidant Activity of alcoholic extract of *Mucuna pruriens* leaves in various methods.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Concentration (µg/ml)</th>
<th>DPPH</th>
<th>NO</th>
<th>% inhibition Lipid peroxidation</th>
<th>Reducing power</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>7.2</td>
<td>6.40</td>
<td>7.9</td>
<td>8.1</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>13</td>
<td>10.40</td>
<td>12.2</td>
<td>10.5</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>18.2</td>
<td>16.14</td>
<td>20.1</td>
<td>17.2</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>27.1</td>
<td>27.33</td>
<td>29.2</td>
<td>22.7</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>35.2</td>
<td>34.10</td>
<td>39.3</td>
<td>29.7</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>42</td>
<td>52.40</td>
<td>56</td>
<td>33.5</td>
</tr>
<tr>
<td>7</td>
<td>200</td>
<td>53</td>
<td>63</td>
<td>68</td>
<td>48.6</td>
</tr>
<tr>
<td>8</td>
<td>400</td>
<td>79</td>
<td>66</td>
<td>78</td>
<td>65.9</td>
</tr>
<tr>
<td>9</td>
<td>800</td>
<td>80</td>
<td>69</td>
<td>85</td>
<td>86.4</td>
</tr>
<tr>
<td>10</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>187.5</td>
<td>97</td>
<td>140</td>
<td>205</td>
</tr>
</tbody>
</table>

(Values are mean of 3 replicates)

Table III Phytochemical analysis of alcoholic extract of *Mucuna pruriens* leaves:

<table>
<thead>
<tr>
<th>TESTS</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+ve</td>
</tr>
<tr>
<td>Proteins</td>
<td>+ve</td>
</tr>
<tr>
<td>Amino acids</td>
<td>+ve</td>
</tr>
<tr>
<td>Steroids</td>
<td>+ve</td>
</tr>
<tr>
<td>Volatile oils</td>
<td>+ve</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+ve</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>+ve</td>
</tr>
</tbody>
</table>

Results of In-vitro antibacterial activity, In-vitro antioxidant activity and phytochemical analysis of alcoholic extract of *Mucuna pruriens* leaves were conducted and given in table.I,II and III.

The anti microbial activity of alcoholic extract of *Mucuna pruriens leaves* was studied by both qualitative and quantitative methods like disc diffusion and cup plate methods against various microorganisms using different concentrations against streptomycin is the standard drug. Disc diffusion method is used extensively to investigate the anti microbial activity of natural substances and plant extracts, these assays are based on the use of discs as reservoirs containing solutions of the substances to be examined in the case of solutions with low activity, however large concentrations or volume added, because of limited capacity of discs,
holes or cylinders are preferably used. Cup plate method is quantitative method to evaluate antimicrobial activity by measuring zone of inhibition. *MP* showed maximum activity at 240 mg/ml against all the bacterial strains and it compete with Streptomycin at 1 mg/ml concentration. Antimicrobial activity of *Mucuna pruriens* may be due to presence of tannins, flavonoids, alkaloids. *In vitro* antioxidant activity of *MP* was carried out in various antioxidant models. Oxidative stress has been implicated in the pathology of many diseases [21]. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals and by many other mechanisms and thus prevents disease [22]. The antioxidant activity is perhaps related to the H⁺ ions donating capability of the extract, which scavenges the peroxyl radical to inhibit or terminate the peroxidation chain. The nitrite produced by the incubation of solution of sodium nitro prusside in standard phosphate buffer at 25° was reduced by alcoholic extract of *MP*. This may be due to the antioxidant principles in the *Mucuna pruriens* leaf extract, which compete with oxygen to react with nitric oxide there by inhibiting the generation of nitrite. The DPPH test provides information on the reactivity of test extract with a stable free radical. DPPH is stable nitrogen centered free radical containing an odd electron on its structure that can accept an electron or hydrogen radical to become a stable diamagnetic molecule and usually utilized for detection of radical scavenging activity [23]. Because of its odd electron DPPH gives a strong absorption at 517 nm in the visible region (deep violet colour). As the electron becomes paired off in presence of a free radical, the absorbance diminishes, thus the resulting decrease in absorbance is Stoichiometric with respect to the number of electrons taken up [24]. The MPE exhibited marked and dose dependent free radical scavenging effect in DPPH radical scavenging assay showing the IC₅₀ value of 187.5µg/ml.

Lipid peroxidation can be prevented either by reducing the formation of free radicals or by supplying the competitive substrate for unsaturated lipids in the membrane or by accelerating the repair mechanisms of damaged cell membrane. Several natural and synthetic antioxidants are used to prevent the lipid peroxidation. lipid peroxidation assay Showing the IC₅₀ value of 140µg/ml [25,26] this activity is perhaps related to the H⁺ ion donating capability of the extract, which scavenges the peroxyl radical to inhibit (or) terminate the peroxidation chain [13]. The antioxidant activity of the *MP* extract was further confirmed by evaluating Nitric Oxide scavenging with IC₅₀ value of 97 ug/ml and Reducing power activity with IC₅₀ value of 205 ug/ml. The *MP* extract effectively scavenged the free radical in NO and Reducing power.
in a dose related manner. The activity is may be due to presence of tannins, flavonoids, alkaloids and amino acids of the *Mucuna pruriens* leaves.

**CONCLUSION**

On the basis of the results obtained in this present investigation, conclude that the alcoholic extract of *Mucuna pruriens* leaves had significant antibacterial activity and antioxidant activity. The obtained results may provide a support to some uses of the plant in traditional medicine. The important findings of the study is that maximum *in vitro* scavenging activity in alcoholic extract of *Mucuna pruriens* leaves. Further studies are recommended to isolate the exact active components responsible for the antimicrobial activity and antioxidant activity.

**REFERENCES**


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