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Study of phytochemical and antimicrobial activity of Alcohlic extract of *Mucuna pruriens* (L.) Leaves

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Abstract
The present paper deals the antioxidant activity of alcoholic extract of leaves of *Mucuna pruriens (MP)* of family Fabaceae. The concentration of alcoholic extract were (3-800 ug/ml). The *MP* showed maximum scavenging activity at concentration of 800 µg/ml. 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*. The antioxidant activity of *MP* and antimicrobial activity may be due to presence of the active principle present in *MP* leaves. 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.

Keywords: *Phytochemical, Mucuna pruriens*, Antibacterial activity and Leaves

Introduction
Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produce these chemicals to protect themselves but recent research demonstrate that they can also protect humans against diseases. There are more than thousand known phytochemicals. Some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soya and flavanoids in fruits. Foods containing phytochemicals are already part of our daily diet. In fact, most foods contain phytochemicals except for some refined foods such as sugar or alcohol. Some foods, such as whole grains, vegetables, beans, fruits and herbs, contain many phytochemicals. It is recommended take daily at least 5 to 9 servings of fruits or vegetable. Fruits and vegetables are also rich in minerals, vitamins and fibre and low in saturated fat.

Phytochemicals are naturally present in many foods but it is expected that through bioengineering new plants will be developed, which will contain higher levels. This would make it easier to incorporate enough phytochemicals with our food.

Mucuna pruriens (Fabaceae) is a typical legume known as velvet bean or Cowitch. names found in Africa, The hindi name is Kewanch. 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 (Kirtikar and Basu, 1980) [1]. The seeds of velvet beans are high in protein, carbohydrates, lipids, fiber, minerals, alkaloids, alkylamines, 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 (Manyam, *et al.* 2004) [2]. It is used in Ayurvedic medicine (Amin, *et al.*1996) [3]. It is used in the prophylactic treatment of snakebites (Tan, *et al.* 2009) [4] 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 (Manyam, *et al.* 2004)[2]. Traditionally, velvet bean has been used as a nerve tonic for nervous system disorders (Katzenchlager, *et al.* 2004)[5]. The antioxidant activity of *Mucuna pruriens* was demonstrated by scavenge DPPH, ABTS and ROS (Guerranti, *et al.* 2008)[6]. *Mucuna pruriens* significantly inhibited the lipids and deoxyribose sugar (Dhanasekaran, *et al.* 2008)[7].

2. Material and Methods

Collection and extraction of plant material

Mucuna pruriens leaves was collected from Govt. Agriculture College experimental fields, in the month of October and plant was identified by the Botanist Dr. A.A. Khan, Retd. Prof. of Botany, Govt. Girls P.G. College, Rewa (M.P.). 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. Rani Durgawati Univ. Jabalpur (M.P.) 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 Botany Deptt. of Govt. Girls P.G. College, Rewa (M.P.). DPPH, sodium nitro-prusside. sulphanilamide, potassium superoxide, O-phosphoric acid, naphthyl, EDTA, KCl, $FeSO_4$, TBA, TCA, BHT, NBT, DMSO, NaOH. Distilled water, peptone, Agar-Agar, Meat extract, NaCl, Ethanol. Some chemicals are also purchased from Gupta Scientific Store Amahiya Road Rewa (M.P.)

Preliminary Phytochemical analysis

Preliminary Phytochemical analysis was carried out according to standard protocol (Kokate, *et al.* 2002) [8].

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

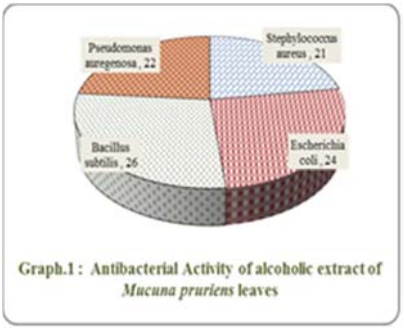
A strain of *Bacillus subtilis* sensitive to streptomycin is employed as the test organism. A stock spore suspension is prepared by cultivation of the organisms on agar or in submerged culture. When microscopic examination reveals good sporulation, the cells are separated from the medium, suspended in 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.

3. Results

Results of antibacterial, antioxidant activity and phytochemical analysis of alcoholic extract of *Mucuna pruriens* leaves were conducted and given in tables 1, 2 and 3.

Table 1: Antibacterial Activity of alcoholic extract of *Mucuna pruriens* leaves

Zones of inhibition (mm)[3]							
	Mucuna pruriens (mg/ml)						Streptomycin (1 mg/ml)
Microorganism	240	160	80	40	20	5	
Stephylococcus aureus	45	15	12	0	0	0	21
Escherichia coli	28	13	0	0	0	0	24
Bacillus subtilis	38	14	12	11	0	0	26
Pseudomonas auregenosa	34	18	17	14	0	0	22

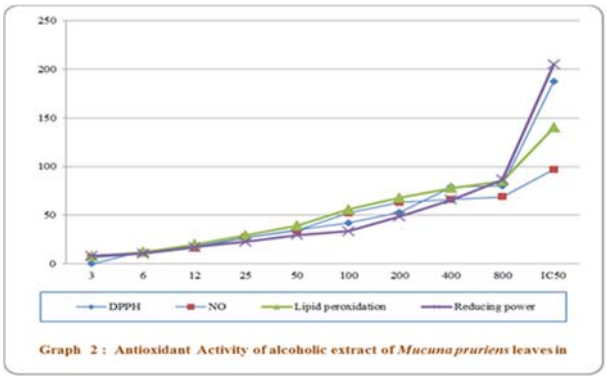


Graph.1 : Antibacterial Activity of alcoholic extract of *Mucuna pruriens* leaves

Table 2: Antioxidant Activity of alcoholic extract of *Mucuna pruriens* leaves in various methods.

S. No.	Concentration (µg/ml)	% inhibition			
		DPPH	NO	Lipid peroxidation	Reducing power
1	3	7. 2	6.40	7.9	8.1
2	6	13	10.40	12.2	10.5
3	12	18.2	16.14	20.1	17.2
4	25	27.1	27.33	29.2	22.7
5	50	35.2	34.10	39.3	29.7
6	100	42	52.40	56	33.5
7	200	53	63	68	48.6
8	400	79	66	78	65.9
9	800	80	69	85	86.4
10	IC50	187.5	97	140	205

(Values are mean of 3 replicates)



Graph 2 : Antioxidant Activity of alcoholic extract of *Mucuna pruriens* leaves in

Table 3: Phytochemical analysis of alcoholic extract of *Mucuna pruriens* leaves

S.No.	Tests	Results
1.	Carbohydrates	+ve
2.	Proteins	+ve
3.	Amino acids	+ve
4.	Steroids	+ve
5.	Volatile oils	+ve
6.	Glycosides	+ve
7.	Flavanoids	+ve
8.	Alkaloids	+ve
9.	Tannins	+ve

The antimicrobial 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 antimicrobial 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 (Marx, 1987) [9]. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals and by many other mechanisms and thus prevents disease (Youdim and Joseph, 2001) [10]. The anti-oxidant 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 (Blois, 1958) [11]. 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 (Oke and Hamburger, 2002) [12]. The MPE exhibited marked and dose dependent free radical scavenging effect in DPPH radical scavenging assay showing the IC_{50} 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 IC_{50} value of 140µg/ml (Yoshikawa, *et al.* 1983 and Valentao, *et al.* 2002) [13, 14], 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 (Ohkawa, *et al.* 1979) [15]. The antioxidant activity of the *MP* extract was further confirmed by evaluating Nitric Oxide scavenging with IC_{50} value of 97 ug/ml and Reducing power activity with IC_{50} 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.

4. 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.

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