

## Phytoconstituent, Toxicity Evaluation and Antiepileptic Activity of Ethanolic Leaf Extract of *Mucuna pruriens*

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### ABSTRACT

Medicinal plants continue to play a crucial role in the treatment of diseases due to their rich phytochemical profiles and pharmacological potential. *Mucuna pruriens* is traditionally used in African ethnomedicine for neurological conditions, including seizures. This study evaluated the phytoconstituents, acute toxicity, and antiepileptic activity of the ethanolic leaf extract of *M. pruriens*. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, phenolics, terpenoids, and cardiac glycosides. Quantitative analysis showed high concentration of alkaloids ( $435.00 \pm 0.00$  mg/g) and phenolics ( $376.67 \pm 2.31$  mg/g), which are known to exert central nervous system (CNS) effects. Acute toxicity testing revealed no mortality up to 5000 mg/kg orally (*p.o.*), classifying the extract as practically non-toxic. Anticonvulsant activity was assessed using pentylenetetrazol (PTZ) and strychnine (STR). The extract completely abolished PTZ-induced seizures (100% protection) and significantly delayed STR-induced convulsions (65% protection), compared to standard drug (diazepam) suggesting modulation of GABAergic and glycinergic pathways. The findings support the ethnomedicinal use of *M. pruriens* in epilepsy management.

**Keywords:** *Mucuna pruriens*, epilepsy, phytochemical screening, toxicity, PTZ, strychnine.

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## INTRODUCTION

Plants have served as a major source of therapeutic agents throughout human history, contributing significantly to global healthcare [1,2]. Their continued use is driven by affordability, availability, and demonstrated efficacy in managing diverse ailments [3]. The relevance of medicinal plants is further supported by the presence of bioactive phytochemicals such as alkaloids, flavonoids, tannins, phenolics, glycosides, steroids, and terpenoids compounds known to exhibit a wide range of pharmacological activities [4, 5,6].

*Mucuna pruriens* (Fabaceae) is used traditionally for treating diabetes, infections, infertility, nervous disorders, inflammation, and convulsive conditions [7,8]. Several studies have reported neuroprotective, antioxidant, and CNS-modulating effects of its seeds and extracts [9,10]. Phytochemicals present in *M. pruriens*, especially alkaloids, phenolics, and flavonoids, have been associated with neurological benefits, including anticonvulsant activities [11,12].

Epilepsy is a chronic neurological disorder characterized by recurrent, unprovoked seizures resulting from abnormal neuronal discharges in the brain <sup>(13)</sup>. It affects approximately 50 million people worldwide, with higher prevalence in developing nations due to limited access to medical care and antiepileptic drugs [14]. In Nigeria, epilepsy remains a major public health concern. Although conventional antiepileptic drugs are available, many are associated with adverse effects, limited efficacy, and drug resistance, necessitating the search for safer alternatives.

The PTZ seizure model induces convulsions through inhibition of GABA<sub>A</sub> receptors thereby reducing inhibitory neurotransmission <sup>(15)</sup>. In contrast, strychnine induces seizures by blocking glycine receptors in the spinal cord and brainstem <sup>(16)</sup>. Evaluating a plant extract in both models provides insight into its potential mechanisms of anticonvulsant action.

Given the ethnomedicinal use of *M. pruriens* in neurological disorders and the presence of bioactive compounds known to modulate CNS function [17,18], this study was designed to investigate the phytochemical constituents, acute toxicity, and antiepileptic activity of the ethanolic leaf extract of *M. pruriens*.

## MATERIALS AND METHODS

### Plant Collection and Identification

Fresh leaves of *M. pruriens* were collected and authenticated at the Department of Pharmacognosy, University of Maiduguri, in accordance with botanical standards [19]. A voucher specimen (UMM/FPH/FAA/014) was deposited. The leaves were cleaned, shade-dried for two weeks to preserve heat-labile phytoconstituents, pulverized, and stored in airtight containers.

### Extraction of Plant Material

Soxhlet extraction was performed on 1800 g of powdered leaves using ethanol, a solvent known to efficiently extract both polar and moderately non-polar phytochemicals [3]. Extraction continued until a clear siphon indicated exhaustion. The extract was concentrated under reduced pressure and dried. Ethanol extraction is widely used because it preserves alkaloids, flavonoids, phenolics, and tannins [20,21].

### Sample collection, Identification and Preparation

Fresh leaf sample of *M. pruriens* was collected from Jos North Local Government Area, Plateau State, Nigeria, and was authenticated by Dr. C.A. Ukwubile (a taxonomist) at the Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Borno State, Nigeria. A voucher specimen number **UMM/FPH/FAA/014** was deposited for the plant at the herbarium of the Department of Pharmacognosy. The sample collected was air-dried under shade and pulverized using wooden mortar and pestle.

### Sample Extraction

One thousand eight hundred grams of dried powdered material was extracted with absolute ethanol using Soxhlet method. After sample has been extracted, the solution was cooled and

filtered to remove the debris with filter paper and muslin cloth, and concentrated on hot air oven at 40°C – 50°C to dryness. The extract was weighed and kept in an air tight container for further analysis.

### Phytochemical Screening

#### Qualitative phytochemical screening

The ethanol fraction of *M. pruriens* was assessed for the presence of some phytochemicals using the standard methods [22,23,24,25,26,27]:

##### Test for Alkaloids

- Dragendorff's reagent: 8 g of Bi (NO<sub>3</sub>.5H<sub>2</sub>O) was dissolved in 20 mL of nitric acid and 2.72 g of potassium iodide in 50 mL of water, mixed and made to 100 mL with distilled water [26,27].

*Test:* To 0.1g of extract, 2.0 mL of HCl was added. To this acidic medium, 1 mL of reagent was also added. An orange-red precipitate produced immediately indicates the presence of alkaloids [25,26,27].

- Wagner's reagent: 1.0 g of iodine and 2.0 g of potassium iodide were dissolved in 5 mL sulphuric acid and the solution was diluted to 100 mL

*Test:* 0.1 g extract was acidified by adding 1.5% v/v HCl and a few drops of

Wagner's reagent. The formation of yellow/brown precipitate confirmed the presence of alkaloids [11,25,26,27].

- Meyer's reagent: 1.36 g Mercuric chloride was dissolved in 60 mL of distilled water and 5 g of potassium iodide in 10 mL of water. The two solutions were mixed and diluted to 100 mL with distilled water.

*Test:* To 0.1g mL of extract, a few drops of reagent was added. The formation of white/ pale precipitate shows the presence of alkaloids [11,25,26,27]

##### Test for flavonoids

Ammonia/H<sub>2</sub>SO<sub>4</sub> Test: The sample was added to 5 mL of diluted ammonia solution and concentrated H<sub>2</sub>SO<sub>4</sub>. The disappearance of the yellow colour on standing indicates the presence of flavonoids [24,26,27].

##### Test for saponins

Frothing Test: 3 mL of the aqueous solution of the extract was mixed, stopped in a test tube and

shaken vigorously for about 30sec, then allowed to stand for 3min and observed for a persistent honeycomb froth, which indicates the presence of saponins [27].

##### Test for tannins

Ferric chloride method: 2 mL of the aqueous extract was added to 3 drops of 10% Ferric chloride solution. The occurrence of a blue-black colour shows the presence of tannins [27].

##### Test for anthraquinone

To 0.1 g of the dried extract was placed in a dry test tube and 5 mL of chloroform was added and heated in a steam bath for 1 minute. The extract was then filtered while hot and allowed to cool. A 10% ammonia solution was also added to the filtrate and then shaken. The upper aqueous layer was observed and there was no bright pink colour which indicates the absence of anthraquinones [27].

##### Test for terpenoids

Salkowski test: 0.1 g of the sample was mixed with 2 mL of CHCl<sub>3</sub> in a test tube. 3 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was also carefully added to the mixture to form a layer. An interface with a reddish-brown colouration was formed if a terpenoid constituent was present [27].

##### Test for cardiac glycosides

A solution of glacial acetic acid (4.0 mL) with 1 drop of 2.0% FeCl<sub>3</sub> was mixed with 10 mL aqueous plant extract and 1 mL concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring formed between the layers shows the presence of cardiac glycosides [26,27].

##### Test for phenol

About 3-4 drops of neutral 5% ferric chloride were added to 1mL of the sample. The formation of a dark green colour indicates the presence of phenols [26,27].

##### Test for steroids

The extracts were added 5 mL of chloroform, shaken and filtered, and a few drops of acetic anhydride along with a few drops of concentrated sulphuric acid was also added through the side of the tube. The appearance of green colour indicates the presence of sterols in the extracts [26,27].

### **Quantitative Phytochemical Screening**

#### **Quantitative Determination of Alkaloids**

Exactly 200 ml of 10% acetic acid in ethanol was added to the sample (5 g) in a 250 ml beaker and allowed to stand for 4 h. The ethanol fraction was concentrated in a water bath to ¼ of its original volume and 10 mL of concentrated ammonium hydroxide was added until the precipitation was complete and filtered after 3 hours with a pre-weighed filter paper. The residues were then dried in an oven [27,28]. The percentage of alkaloid was calculated using the formula below:

$$\% \text{ alkaloids} = \frac{x}{y} \times 100 \quad (\text{i})$$

Where x = weight of alkaloid and y = weight of extract used.

#### **Determination of Tannin Content**

Tannins contents were quantitatively determined by the Folin-Ciocalteu method for ethanol fraction. 0.1 mL of sample was added to volumetric flasks (10 mL) both containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu phenol reagent, 1 mL of 35 %  $\text{Na}_2\text{CO}_3$  solution was diluted to 10 mL with distilled water. The mixtures were shaken well and kept at room temperature for 30 minutes. Reference standard solutions of gallic acid (32, 63, 125, 250, 500, and 1000  $\mu\text{g}/\text{mL}$ ) were also prepared. Absorbance for test and standard solutions was measured against the blank at 760 nm with a UV/Visible spectrophotometer [24, 27].

#### **Determination of Total Flavonoid Content**

Total flavonoid content was determined from the calibration curve of quercetin and expressed as a microgram of quercetin equivalent per milligram of extract. Total flavonoid content was determined according to the procedure of [24], validated by [29], with some modifications. 1 mg/1 mL standard solution was prepared, and series concentrations of quercetin were then obtained. The sample solution (0.5mL) was added with 1.5 mL ethanol, 0.1 mL of 10%  $\text{AlCl}_3$ , 0.1 mL Potassium acetate 1M, and 2.8 mL of distilled water, and then incubated for 30 minutes. Absorbance was also measured at 415 nm. Distilled water was used as blank. Total flavonoid content was expressed in mg quercetin per 1g of plant extract [27,28].

### **Total Phenolic Content**

The total phenolic content of ethanol extract (200-1000  $\mu\text{g}/\text{mL}$ ) was quantified using the Folin-Ciocalteu's phenol reagent with gallic acid (50-200  $\mu\text{g}/\text{mL}$ ) as standard. The plant sample (1 mL) was added to 1 mL of Folin-Ciocalteu's reagent (diluted ten-fold in distilled water) in separate test tubes. The content of each test tube was mixed and allowed to stand for five minutes at 25°C in an incubator. One mL of 2 % sodium carbonate solution ( $\text{Na}_2\text{CO}_3$ ) was added to the mixture. This was allowed to stand for 2 h at 25°C in an incubator and centrifuged at 1000 g for 10 minutes to get a clear solution. The absorbance of the supernatant was then determined at 760 nm using a UV spectrophotometer. Distilled water (1 mL) was also added to 1 mL Folin-Ciocalteu's reagent (diluted ten-fold in distilled water) and processed in the same way as done for the test samples and used as blank. All measurements were done in triplicates. The total phenolics were expressed as micrograms per millilitre of gallic acid equivalents through the calibration curve with gallic acid [27,28,29].

#### **Experimental Animals and Ethical Approval**

Adult Wistar rats (150-200 g) were obtained and acclimatized for 14 days under standard laboratory conditions (12 h light/dark cycle, controlled temperature), with free access to food and water. Housing and care complied with international animal welfare guidelines for laboratory research and phytomedicine studies involving rodents [14].

**Ethical approval:** All procedures were approved by the University of Maiduguri Animal Ethics Committee, in accordance with institutional guidelines.

#### **Acute Oral Toxicity ( $\text{LD}_{50}$ ) Study**

The acute toxicity investigation was carried out using Lorke's method [29,30]. In this study, one route of administration was used, that is, oral (*p.o.*). In phase I, rats were divided into three groups of three rats each and were treated with the extracts being dissolved in normal saline at doses of 10, 100 and 1000 mg/kg body weight (*p.o.*) observed for 24 h for mortality to determine the dose range. In phase II, rats were divided into

three groups of three rats each and the extracts, being dissolved in normal saline were administered at 1600, 2900 and 5000 mg/kg body weights (*p.o.*). Recovery and weight gain were considered as signs that the acute toxicity had passed. The LD<sub>50</sub> of *M. pruriens* was determined at the Department of Veterinary Pharmacology and Toxicity, Faculty of Veterinary Medicine, University of Maiduguri Nigeria. The value of the LD<sub>50</sub> was calculated with the formula below:

$$LD_{50} = \sqrt{(a \times b)} \quad (ii)$$

Where a = Highest value that gave no mortality and b = Lowest dose that produced mortality.

#### Antiepileptic Evaluation of Plant Extract

##### Pentylenetetrazol (PTZ) Induced Convulsion

The animals (Wistar rats) were divided into five groups of five animals each (n=5). The animals were treated for three days as follows: Group 1: normal saline, Group 2: diazepam (5 mg/kg), Group 3: EtF (100 mg/kg), Group 4: EtF (200 mg/kg), Group 5: EtF (400 mg/kg). One hour after the given treatment on the third day, convulsion was induced chemically by administering PTZ (80 mg/kg) as reported previously by [15] with slight modification. The animals were monitored individually in a plastic cage for 30 minutes with the animal that survived up to 24 hours. Some data such as Latency, the onset of tonic convulsions and % protection were determined [14]. The percentage inhibition of epilepsy was calculated with the formula:

$$\% \text{ Inhibition} = \frac{x-y}{x} \times 100 \quad (iii)$$

where x = convulsion in the control group and y = convulsion in the treated group.

##### Strychnine (STR) Induced Convulsion

The animals (Wistar rats) were divided into five groups of five animals per group. The animals were treated for three days as follows: Group 1: received 10 mL of normal saline, group 2 received diazepam (5 mg/kg), group 3 received 100 mg/kg b.w. of EtF, group 4 received 200 mg/kg b.w. of EtF, and group 5 received 400 mg/kg b.w. of EtF. One hour after the given treatment on the third day, convulsion was induced chemically by administering STR (3.5 mg/kg) as previously reported by <sup>(1)</sup> with slight modification. The animals were monitored individually in a plastic cage for 30 minutes with the animals that survived up to 24 hrs. Some data such as Latency,

the onset of tonic convulsions and % protection were determined [16].

#### Statistical Analysis

Data were presented as mean  $\pm$  SD. Statistical significance was analysed by ANOVA, followed by Dunnett's post hoc test ( $p < 0.05$ ), consistent with standard pharmacological evaluations <sup>(26)</sup>.

#### RESULTS AND DISCUSSION

The results of extraction profile presented in (Table 1) showed percentage yield of 18.1 % (33.5g w/w) and this indicates that ethanol is a good solvent for extraction. The reddish-brown extract is consistent with previous reports on ethanol extraction of *M. pruriens* leaves [31]. Which also indicates the presence of phenolics, flavonoids and tannins [31].

Phytochemical analysis of the ethanolic leaf extract of *M. pruriens* showed the presence of alkaloids, flavonoids, phenolics, cardiac glycosides, terpenoids, tannins and carbohydrates (Table 2) which is also in agreement with the previous studies carried out by [3,7,11,32]. The abundant presence of alkaloids and phenolics is notable, as these compounds have documented neuroprotective and anticonvulsant activities [18, 26]. Ethanol leaf extract (EtF) contained high concentrations of alkaloids and phenolics (Table 3). These classes are well known for CNS depressant, antioxidant, and membrane-stabilizing activities [23,33].

The extract demonstrated excellent safety, with no mortality recorded up to 5000 mg/kg (Table 4), placing it in the category of practically non-toxic substances [35]. Studies on medicinal plants with similar phytochemical composition often show wide safety margins, especially when phenolics and flavonoids predominate <sup>(26)</sup>. Mild transient behavioural changes at high doses are not uncommon for CNS-active plant extracts <sup>(26)</sup> and do not indicate significant toxicity. Transient behavioural signs at very high doses (e.g., reduced locomotion) are typical for plant extracts with CNS activity [36].

The extract exhibited complete abolition of PTZ-induced convulsions (Table 5), strongly suggesting potentiation of GABAergic inhibitory

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neurotransmission. PTZ induces seizures by antagonizing GABA<sub>A</sub> receptors, reducing inhibitory chloride influx <sup>(15)</sup>. Therefore, the complete prevention of PTZ-induced seizures at all tested doses indicates that the extract may enhance GABA-mediated neuronal inhibition. This is consistent with earlier observations that alkaloids and flavonoids can modulate GABA<sub>A</sub> receptors [31]. The effect of EtF was comparable to diazepam, a classical GABA<sub>A</sub> modulator, reinforcing this mechanism.

In the strychnine model, EtF significantly delayed seizure onset and reduced convulsion duration (Table 6). Since strychnine blocks glycine receptors in the spinal cord and brainstem [7], the ability of the extract to suppress strychnine-induced convulsions suggests additional modulation of glycinergic inhibitory pathways. This broader spectrum of action indicates the presence of multiple active constituents acting synergistically. Phenolics and

tannins, which have membrane-stabilizing and antioxidant effects [37], may contribute to this activity by reducing excitotoxic neuronal firing. The reduction in convulsion duration may relate to phenolic antioxidant activity that reduces excitotoxicity [18]. EtF produced significant dose-dependent increases in seizure latency and reductions in convulsion duration, suggesting possible glycinergic modulation [7].

Overall, the combined results from PTZ and STR models suggest that the extract possesses broad-spectrum anticonvulsant properties, likely mediated through enhancement of both GABAergic and glycinergic neurotransmission, as well as through antioxidant mechanisms. This aligns well with the ethnomedicinal use of *M. pruriens* in treating convulsive and neurological disorders [7, 38]. Similar findings have been reported for related medicinal plants rich in alkaloids and phenolics [12].

**Table 1: Extraction profile of ethanol leaf extract of *Mucuna pruriens***

Extract Mass (g w/w)	% Yield	Colour	Texture
EtF 32.5	18.1	Reddish brown	Amorphous

**Table 2: Qualitative phytochemical constituents of ethanol leaf extract (EtF) of *Mucuna pruriens***

Phytochemical	EtF
Alkaloids	+
Flavonoids	+
Tannins	+
Phenolics	+
Cardiac glycosides	+
Terpenoids	+
Saponins	-
Steroids	-
Anthraquinones	-

**Table 3: Quantitative phytochemical constituents of ethanol leaf extract (EtF) of *Mucuna pruriens***

Phytochemical	EtF (mg/g)
Total alkaloids	435.00 ± 0.00
Total tannins (mgGAE/g)	88.00 ± 3.46
Total flavonoids (mgQE/g)	1.00 ± 0.00
Total phenolics (mgGAE/g)	376.67 ± 2.31

Key: ethanol leaf extract (EtF)

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**Table 4: Acute oral toxicity of ethanol leaf extract (EtF) of *Mucuna pruriens* (Lorke's test)**

Dose (mg/kg)	Mortality
10	0/3
100	0/3
1000	0/3
1600	0/3
2900	0/3
5000	0/3

LD<sub>50</sub> > 5000 mg/kg

**Table 5: Effect of ethanol leaf extract (EtF) on PTZ-induced convulsion**

Dose (mg/kg)	Convulsion Duration (sec)	% Protection
Normal saline (Control)	491.20 ± 7.54	–
100 mg/kg	0.00 ± 0.00	100
200 mg/kg	0.00 ± 0.00	100
400 mg/kg	0.00 ± 0.00	100
Standard drug (Diazepam)	0.00 ± 0.00	100

Results are expressed as means ± SD (n = 5). \* Statistically significant at p < 0.05 (one-way)

**Table 6: Effect of ethanol leaf extract (EtF) on STR-induced convulsion**

Dose (mg/kg)	Seizure Latency (Sec.)	Convulsion (Sec.)	% Protection
Normal saline (Control)	–	472.40 ± 12.48	–
100	78	212.20 ± 10.41*	55.08 ± 4.55
200	108	208.60 ± 5.33*	55.84 ± 4.66
400	162	161.20 ± 16.01*	65.88 ± 7.19
Standard Drug (Diazepam)	–	0.00 ± 0.00	100.00 ± 0.00

Results are expressed as means ± SD (n = 5). \* Statistically significant at p < 0.05 (one-way)

## RECOMMENDATIONS

Conduct chronic toxicity studies including liver and kidney function assays.  
Perform bioassay-guided fractionation to isolate the most active components.  
Investigate antioxidant, enzyme inhibitory, and receptor-binding activities.  
Explore effects on cognitive and behavioural parameters in seizure-induced models.  
These steps will provide a clearer understanding of the therapeutic potential and mechanism of action of *M. pruriens*.

## CONCLUSION

The ethanolic leaf extract of *Mucuna pruriens* contains significant levels of alkaloids, phenolics, tannins, and flavonoids; phytochemicals known

for their neuroprotective and anticonvulsant effects. The extract is safe at high doses (LD<sub>50</sub> > 5000 mg/kg) and demonstrated potent anticonvulsant activity by completely abolishing PTZ-induced seizures and significantly reducing STR-induced convulsions. These results strongly support the traditional use of *M. pruriens* in the management of epilepsy and suggest its potential as a natural source for developing safer anticonvulsant agents.

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