Pharmacology & Pharmacy, 2026, 17(11), 317-337
https://www.scirp.org/journal/pp
ISSN Online: 2157-9431
ISSN Print: 2157-9423

Biological Activities, Acute Toxicity and Antimicrobial Resistance Gene Profiling of *Mucuna pruriens* (L.) Seeds Marketed in Guinea-Conakry

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How to cite this paper: Michodjehoun, C., Chokki, S., Konkon, A.K., Akotegnon, R.A., Bah, F., Abatti, F.B.T., Kohonou, A.N., Lehmane, H.T., Sokohou, A., Traore, M.S., Gbago, O. and Alohoutade, S.V. (2026) Biological Activities, Acute Toxicity and Antimicrobial Resistance Gene Profiling of *Mucuna pruriens* (L.) Seeds Marketed in Guinea-Conakry. *Pharmacology & Pharmacy*, **17**, 317-337.
https://doi.org/10.4236/pp.2026.1711018

Received: June 9, 2026
Accepted: November 14, 2026
Published: November 17, 2026

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Abstract

Mucuna pruriens (L.) DC is a medicinal plant widely used in traditional medicine for its therapeutic properties. It is rich in bioactive compounds that may contribute to antimicrobial, antioxidant, and pharmacological activities. However, scientific data regarding its biological potential, toxicity profile, and antimicrobial effectiveness against multidrug-resistant bacteria remain limited. This study aimed to evaluate the phytochemical composition, antibacterial activity, and acute toxicity of aqueous and ethanolic extracts of *Mucuna pruriens* seeds, as well as to investigate associated bacterial resistance genes. The seeds of *Mucuna pruriens* were extracted by maceration using distilled water and ethanol as solvents. The extracts were subjected to qualitative phytochemical screening to identify major secondary metabolites. Antibacterial activity was evaluated against several multidrug-resistant bacterial strains by measuring inhibition zone diameters. Acute toxicity was assessed in Wistar rats at a single oral dose of 2000 mg/kg body weight, following OECD guidelines. Hematological and biochemical parameters were analyzed after treatment. Molecular detection of resistance genes (TEM, SHV, Carba, qnrA in *Salmonella* spp. and mecA in *Staphylococcus aureus*) was performed using standard molecular techniques. The ethanolic extract showed a higher extraction yield (42.18%)

compared to the aqueous extract (14.52%). Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, gallic tannins, mucilages, reducing compounds, and steroid-terpenes. Antibacterial testing demonstrated variable sensitivity among bacterial strains, with the ethanolic extract exhibiting stronger activity than the aqueous extract against several multidrug-resistant isolates. No mortality was observed in rats treated with a single dose of 2000 mg/kg, indicating low acute toxicity. However, hematological and biochemical variations were observed, particularly in the ethanolic extract group, suggesting mild physiological effects. Molecular analysis revealed the presence of the TEM gene in several *Salmonella* spp. strains, while SHV, Carba, and qnrA genes were absent. In *Staphylococcus aureus*, the mecA gene was detected in one clinical strain (S6), indicating methicillin resistance, while it was absent in the reference strain. *Mucuna pruriens* seeds demonstrate promising biological potential, particularly in terms of antibacterial activity and phytochemical richness, with relatively low acute toxicity. However, observed hematological and biochemical changes, especially with the ethanolic extract, highlight the need for further studies to better understand its safety profile and mechanisms of action. This plant may represent a valuable source of bioactive compounds for future therapeutic applications against infectious diseases and antimicrobial resistance.

Keywords

Mucuna pruriens, Antibacterial Activity, Phytochemistry, Acute Toxicity, Antimicrobial Resistance

1. Introduction

Natural products remain an important source of bioactive compounds for the development of pharmaceutical, nutraceutical, food, and cosmetic products. Medicinal plants are particularly valued because they contain a wide variety of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds, which have been associated with numerous biological activities such as antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and neuroprotective effects. Consequently, plant-derived products continue to attract considerable scientific interest as potential alternatives or complementary approaches to conventional therapies [1]-[3].

Among these medicinal plants, *Mucuna pruriens* (L.) DC., a tropical legume belonging to the Fabaceae family, has attracted increasing attention due to its nutritional and therapeutic value. The species is widely distributed in Africa, Asia, and Latin America, where it is traditionally used for the management of various health conditions and as a nutritional supplement [4] [5]. The seeds are particularly rich in proteins, essential amino acids, phenolic compounds, flavonoids, alkaloids, and L-DOPA (L-3,4-dihydroxyphenylalanine), a natural precursor of dopamine extensively used in the treatment of Parkinson's disease [6] [7].

Several studies have reported diverse biological activities of *Mucuna pruriens*, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, neuroprotective, anxiolytic, and antidepressant properties [8]-[10]. These effects are mainly attributed to the presence of bioactive phytochemicals capable of modulating oxidative stress, inflammation, and microbial growth. However, the phytochemical composition and biological activities of medicinal plants may vary according to environmental conditions, geographical origin, cultivation practices, harvesting period, storage conditions, and extraction methods. Therefore, local investigations remain essential to validate the biological potential and safety of plant materials available in specific regions.

Simultaneously, antimicrobial resistance has emerged as one of the most significant threats to global public health. The increasing prevalence of multidrug-resistant bacterial pathogens has reduced the effectiveness of many conventional antibiotics, leading to prolonged illness, increased healthcare costs, and higher mortality rates [11] [12]. Resistance is frequently associated with the acquisition of specific genetic determinants, including β-lactamase genes such as TEM and SHV, carbapenemase genes, plasmid-mediated quinolone resistance genes such as qnrA, and the mecA gene responsible for methicillin resistance in *Staphylococcus aureus*. The detection of these resistance markers is essential for understanding bacterial resistance profiles and evaluating the potential usefulness of alternative antimicrobial agents [13] [14].

In Guinea-Conakry, *Mucuna pruriens* seeds are commonly commercialized in local markets and widely used by the population for nutritional and traditional medicinal purposes. Despite their widespread use, limited scientific information is available regarding the phytochemical composition, antibacterial activity, toxicological profile, and potential relationship between the biological activity of locally marketed *Mucuna pruriens* seeds and the resistance characteristics of clinically relevant bacterial strains. Furthermore, few studies have investigated the occurrence of antimicrobial resistance genes in bacterial isolates used to evaluate the antibacterial activity of this plant.

Therefore, the present study aimed to investigate the phytochemical composition, antibacterial activity, acute oral toxicity, and antimicrobial resistance gene profiles associated with aqueous and ethanolic extracts of *Mucuna pruriens* seeds marketed in Guinea-Conakry. Specifically, the study sought to determine extraction yields, identify major phytochemical constituents, evaluate antibacterial activity against multidrug-resistant bacterial strains, assess acute oral toxicity in Wistar rats following OECD guideline 423, and detect selected resistance genes (TEM, SHV, Carba, qnrA, and mecA) in the tested bacterial isolates.

2. Materials and Methods

2.1. Study Area

All experimental work related to this study was conducted at the Laboratory of Nutritional Sciences, Department of Food Science and Nutrition, ESTH, Cona-

kry, Guinea, which served as the main research site for all experimental procedures, including sample processing, phytochemical analyses, and microbiological assays. This laboratory is a key institution dedicated to research in food science, applied nutrition, and the study of bioactive compounds from natural sources.

This work was carried out in collaboration with the Institute of Applied Biology Research of Guinea (IRBAG), Kindia, Guinea, a national reference institution dedicated to biomedical research, the valorization of natural substances, applied nutrition, and the study of interactions between diet and human health.

2.2. Materials

2.2.1. Plant Material

Seeds of *Mucuna pruriens* (L.) DC. were purchased from five independent vendors located in the Madina, Niger, and Matoto markets in Conakry, Guinea. The plant material was authenticated by Dr. Konkon Alphonse, a researcher at the Institute of Applied Biology Research of Guinea (IRBAG), where a voucher specimen (No. MP-2025-04) was deposited in the herbarium for future reference.

To ensure sample representativeness, seeds from the different vendors were pooled, then sorted and cleaned to remove debris and damaged materials. The seeds were air-dried at room temperature, ground into a fine powder using an electric grinder, and stored in airtight containers protected from light and moisture until further use [15].

2.2.2. Animal Material

The animal material consisted of male and female Wistar albino rats weighing between 120 and 180 g. The animals were acclimatized for two weeks in standard cages, with free access to food and water, under controlled temperature (22˚C ± 2˚C) and a 12 h light/dark cycle [16].

2.2.3. Extraction Materials

The materials used for extraction included: *Mucuna pruriens* seed powder, distilled water, 96% ethanol, precision balance, beakers, Erlenmeyer flasks, magnetic stirrer, filter paper, oven, and DMSO (dimethyl sulfoxide) [17].

2.2.4. Microbiological Materials

Microbiological equipment included Petri dishes, Mueller-Hinton agar, micropipettes, sterile tips, swabs, incubator, Falcon tubes, inoculating loop, and Bunsen burner [18].

2.2.5. Bacterial Strains

Antibacterial tests were performed on multidrug-resistant strains isolated from hospital and community settings, including *Staphylococcus aureus* (resistant), ESBL-producing *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella* spp. A reference ATCC strain was used for quality control [19].

2.3. Study Methodology

2.3.1. Preparation of Extracts

For the aqueous extract, 50 g of *Mucuna pruriens* seed powder was weighed and mixed with 500 mL of distilled water. The mixture was macerated for 72 hours under agitation, then filtered. The filtrate was concentrated in an oven at 45˚C and stored in a refrigerator [20].

For the ethanolic extract, 50 g of powder was mixed with 500 mL of ethanol. After maceration for 72 hours, the mixture was filtered. The filtrate was evaporated at 45˚C and stored under refrigeration until use [21].

2.3.2. Extraction Yield

The extraction yield was calculated using the following formula:

$$R(\%) = \frac{M_e}{M_v} \times 100$$

where:

- $R(\%)$ = extraction yield;
- M_e = mass of dry extract obtained;
- M_v = mass of plant material used (22).

2.3.3. Phytochemical Screening

Aqueous and ethanolic extracts were subjected to qualitative phytochemical screening to detect the presence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, polyphenols, terpenes, steroids, coumarins, and mucilages [22].

These analyses were carried out using colorimetric and precipitation reactions as described by Houghton and Raman (1998), allowing preliminary identification of bioactive compounds present in the extracts [23].

2.3.4. Evaluation of Antibacterial Activity

1) Preparation of Inoculum

Bacterial strains were subcultured on Mueller-Hinton agar and incubated at 37˚C for 18 - 24 hours. A bacterial suspension equivalent to 0.5 McFarland standard was prepared [24].

2) Agar Well Diffusion Method

After inoculation of Petri dishes, wells of 6 mm diameter were made in the agar. Each well was filled with 50 µL of plant extract at concentrations of 100 mg/mL and 50 mg/mL. Dimethyl sulfoxide (DMSO) was used as a negative control, while ciprofloxacin (10 µg/disc) was used as a positive control [25].

The plates were incubated at 37˚C for 24 hours. Antibacterial activity was assessed by measuring the diameter of inhibition zones (in mm) around the wells [26].

All antibacterial assays were performed in triplicate (n = 3) under identical experimental conditions. Results were expressed as mean inhibition zone diameter ± standard deviation (SD).

2.3.5. Acute Toxicity Test

The acute oral toxicity test was conducted according to OECD guideline 423. Three groups of rats were used: a control group, one group receiving the aqueous extract at a dose of 2000 mg/kg body weight, and another group receiving the ethanolic extract at the same dose [27]. Each experimental group consisted of six rats (n = 6 per group). The same animals were followed and evaluated at day 0 (baseline) and day 14, allowing intra-individual comparison over time.

Animals were closely monitored for 14 days for signs of toxicity, including mortality, agitation, diarrhea, weight loss, neurological disorders, and skin or fur alterations [28].

2.3.6. Biochemical and Hematological Analyses

At the end of the experiment, blood samples were collected to assess biochemical and hematological parameters.

Biochemical parameters included urea, creatinine, ALT, and AST.

Hematological parameters included red blood cells, white blood cells, hemoglobin, hematocrit, and platelets [29].

2.4. Statistical Analysis

Data were entered into Excel and analyzed using GraphPad Prism or SPSS software. Results were expressed as mean ± standard deviation.

Comparisons between groups were performed using Student's t-test or ANOVA, with a significance level set at $p < 0.05$ [30].

3. Results

3.1. Extraction Yields

The extraction yields of *Mucuna pruriens* (L.) DC. seeds were determined using two solvents with different polarities, namely distilled water and ethanol.

The results obtained showed a yield of 14.52 ± 0.23% for the aqueous extract and 42.18 ± 0.33% for the ethanolic extract. These results indicate that the ethanolic extract has a significantly higher yield than the aqueous extract ($p < 0.05$).

This difference can be explained by the better ability of ethanol to extract a wide range of bioactive compounds present in *Mucuna pruriens* seeds, particularly compounds of intermediate polarity. However, extraction yield also depends on several factors such as the nature of the plant material, harvesting conditions, storage duration, and the extraction method used.

3.2. Phytochemical Potential

The qualitative phytochemical analysis of the powdered *Mucuna pruriens* seeds revealed the presence of several groups of secondary metabolites, including alkaloids, flavonoids, saponins, tannins, mucilages, reducing compounds, as well as steroids-terpenes.

These results are generally consistent with those reported by several authors who have worked on species of the *Mucuna* genus, confirming the chemical richness

of this plant in bioactive compounds. However, some variations may be observed depending on geographical origin, ecological conditions, and the extraction methods used (Table 1).

Table 1. Results of phytochemical screening of *Mucuna pruriens* seeds.

Metabolites	Results
Catechin tannins	–
Gallic tannins	+
Flavonoids	+
Anthocyanins	–
Leuco-anthocyanins	–
Alkaloids	+
Mucilages	+
Reducing compounds	+
Steroids-terpenes	+
Saponins	+
Quinonic derivatives	–

+: Present; –: Absent.

3.3. Antibacterial Activity

The antibacterial activity of the aqueous and ethanolic extracts of *Mucuna pruriens* was evaluated against different multidrug-resistant bacterial strains. The susceptibility of the strains was determined by measuring the diameters of inhibition zones (Table 2).

Table 2. Antibacterial effect of *Mucuna pruriens* extracts on tested bacterial strains.

Strains	AE 100 mg/mL	AE 50 mg/mL	EE 100 mg/mL	EE 50 mg/mL	Ciprofloxacin (10 µg)
P9	0	0	0	0	18.0 ± 0.5
P14	0	0	0	0	17.5 ± 0.4
P15	6.0 ± 0.3	5.0 ± 0.2	8.0 ± 0.4	7.0 ± 0.3	19.0 ± 0.5
P16	0	0	5.0 ± 0.3	4.0 ± 0.2	16.8 ± 0.4
P17	7.0 ± 0.4	6.0 ± 0.3	9.0 ± 0.5	8.0 ± 0.4	20.0 ± 0.6
P19	0	0	0	0	18.2 ± 0.5
P29	8.0 ± 0.5	6.0 ± 0.3	10.0 ± 0.5	9.0 ± 0.4	21.0 ± 0.6
P70	5.0 ± 0.2	0	7.0 ± 0.3	6.0 ± 0.3	17.0 ± 0.4
P362	0	0	6.0 ± 0.3	5.0 ± 0.2	18.0 ± 0.5
P368	7.0 ± 0.4	5.0 ± 0.3	9.0 ± 0.5	8.0 ± 0.4	20.5 ± 0.6
S. aureus ATCC 25923	9.0 ± 0.5	7.0 ± 0.2	12.0 ± 0.6	10.0 ± 0.5	22.0 ± 0.6
S6	6.0 ± 0.3	5.0 ± 0.2	8.0 ± 0.4	7.0 ± 0.3	19.0 ± 0.5

The results showed inhibition diameters ranging from 0 to 12 mm for the plant extracts, indicating variable antibacterial activity depending on the tested strains. Overall, the tested extracts exhibited antibacterial activity ranging from weak to moderate, depending on the bacterial strain and the concentration used.

Analysis of Table 2 shows that *Mucuna pruriens* extracts exhibit antibacterial activity that varies according to the bacterial strain, the type of extract, and the concentration. Overall, the ethanolic extract (EE) demonstrated higher activity than the aqueous extract (AE), with generally larger inhibition zone diameters.

The highest activities were observed with the ethanolic extract at 100 mg/mL (Y1), particularly against strains P29 (10 mm), P17 (9 mm), P368 (9 mm), P15 (8 mm), S6 (8 mm), and the reference strain *Staphylococcus aureus* ATCC 25923 (12 mm). This suggests that ethanol is more efficient in extracting bioactive compounds responsible for antibacterial activity.

The aqueous extract also exhibited antibacterial activity, but to a lesser extent. The largest inhibition zones obtained with AE at 100 mg/mL (X1) were observed for P29 (8 mm), P17 (7 mm), P368 (7 mm), and the ATCC strain (9 mm). At 50 mg/mL, the activity generally decreased, indicating a dose-dependent effect.

Some bacterial strains, particularly P9, P14, and P19, showed no sensitivity (0 mm inhibition zone) to either extract at any tested concentration. This may reflect intrinsic or acquired resistance of these strains to the bioactive compounds present in the extracts.

Since the negative control (DMSO) showed no inhibition zone (0 mm), the observed antibacterial effects can be attributed to the plant extracts rather than the solvent.

For comparison, the positive control (ciprofloxacin) exhibited strong antibacterial activity with inhibition zones ranging from approximately 16.8 to 22.0 mm, confirming the validity of the experimental assay.

Overall, these results indicate that *Mucuna pruriens* possesses promising antibacterial potential, particularly in its ethanolic extract form, supporting further studies aimed at isolating and characterizing the active compounds responsible for this activity (Table 2).

3.4. Acute Toxicity of *Mucuna pruriens* Extracts

3.4.1. LD_{50} of the Extracts

No mortality was observed in rats administered aqueous and ethanolic extracts of *Mucuna pruriens* at a single dose of 2000 mg/kg body weight, suggesting low acute toxicity under the experimental conditions.

3.4.2. Hematological Parameters

Comparative analysis of hematological parameters between the beginning (J0) and the end (J14) of the experiment revealed some variations depending on the group, with more pronounced changes in the group treated with the ethanolic extract (EE) (Table 3).

In the control group, erythrocyte parameters remained generally stable between J0 and J14, indicating normal physiological progression of the animals during the

study. No significant changes were observed in red blood cell count (RBC), hemoglobin (Hb), or hematocrit (HCT).

Table 3. Effect of selected plant extracts at a dose of 2000 mg/kg on blood count parameters.

Group	Day	RBC	WBC	Hb	HCT	MCH	MCHC
C	J0	6.54 ± 1.14	5.06 ± 6.90	13.87 ± 2.86	41.33 ± 7.28	32.20 ± 20.35	33.43 ± 1.04
C	J14	6.67 ± 0.42	0.76 ± 0.70	14.37 ± 0.86	37.80 ± 3.83	21.50 ± 0.53	38.07 ± 1.66
AE	J0	5.85 ± 0.56	7.40 ± 0.79	12.17 ± 0.42	37.83 ± 2.20	20.87 ± 1.38	32.20 ± 0.96
AE	J14	5.89 ± 0.66	11.00 ± 1.91	12.87 ± 1.64	34.00 ± 4.69	21.77 ± 0.40	37.87 ± 1.28*
EE	J0	6.38 ± 0.20	7.23 ± 3.02	13.50 ± 0.43	43.70 ± 1.57	21.17 ± 0.40	30.97 ± 0.11
EE	J14	5.41 ± 0.16*	7.20 ± 2.40	12.70 ± 0.36*	33.47 ± 2.74*	22.87 ± 0.75*	38.10 ± 0.80*

C: Control; AE: Aqueous Extract; EE: Ethanolic Extract; RBC: Red Blood Cells; WBC: White Blood Cells; Hb: Hemoglobin; HCT: Hematocrit; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; *$p < 0.05$, indicating a statistically significant difference.

In the group treated with the aqueous extract (AE), red blood cell counts remained relatively constant between J0 (5.85 ± 0.56) and J14 (5.89 ± 0.66), with no significant difference. Hemoglobin and hematocrit showed a slight, non-significant decrease. However, mean corpuscular hemoglobin concentration (MCHC) increased significantly at J14, suggesting a modification in hemoglobin content within erythrocytes.

In animals treated with the ethanolic extract (EE), a significant decrease in red blood cell count was observed between J0 (6.38 ± 0.20) and J14 (5.41 ± 0.16). Hemoglobin and hematocrit also decreased significantly, indicating a more pronounced effect of this extract on the erythrocyte lineage. However, MCHC increased significantly at J14, suggesting a possible compensatory mechanism through a relative enrichment of hemoglobin in the remaining red blood cells.

3.4.3. Biochemical Parameters

The results of the biochemical analyses showed significant variations ($p < 0.05$) depending on the parameters studied.

Analysis of the biochemical parameters (Table 4) highlights variations among the experimental groups after 14 days of treatment, reflecting differential effects of the aqueous and ethanolic extracts administered at a dose of 2000 mg/kg body weight on renal and hepatic functions.

In the control group, a significant increase in creatinine and AST was observed at J14. These changes may be related to the physiological evolution of the animals or to experimental factors independent of the treatments. In contrast, urea and ALT

did not show any significant variation.

Table 4. Effect of extracts on renal and hepatic parameters.

Group	Day	Urea (mg/L)	Creatinine(mg/L)	AST(UI/L)	ALT(UI/L)
Control	J0	0.83 ± 0.12	7.89 ± 1.14	255.33 ± 20.03	63.45 ± 20.91
Control	J14	0.63 ± 0.03	12.51 ± 0.93*	334.33 ± 23.29*	142.57 ± 35.07
Aqueous extract	J0	0.68 ± 0.13	6.44 ± 0.03	257.33 ± 38.00	83.32 ± 13.62
Aqueous extract	J14	0.57 ± 0.24*	10.14 ± 3.72	365.50 ± 38.53	131.45 ± 15.85*
Ethanolic extract	J0	0.76 ± 0.50	4.95 ± 1.45	226.33 ± 171.00	84.81 ± 7.38
Ethanolic extract	J14	0.60 ± 0.05	11.41 ± 1.64*	321.33 ± 113.56*	127.46 ± 2.25

*$p < 0.05$, indicating a statistically significant difference.

In animals treated with the aqueous extract at 2000 mg/kg, a significant decrease in urea was recorded at J14, suggesting an influence of this extract on nitrogen metabolism or improved renal elimination. Additionally, a significant increase in ALT was observed, which may indicate mild hepatic stress. Creatinine and AST levels increased but did not reach statistical significance.

In the group treated with the ethanolic extract at 2000 mg/kg, a significant increase in creatinine and AST was noted at J14. These results suggest a more pronounced effect of this extract on renal function and on hepatocellular or muscular integrity. However, urea and ALT did not show significant variation.

3.5. Identification of Resistance Genes

3.5.1. *Salmonella* spp. Strains

The molecular analysis of *Salmonella* spp. strains revealed a predominance of the TEM gene, while the SHV, Carba, and qnrA genes were not detected in any of the studied isolates (**Table 5**).

Table 5. Distribution of resistance genes in *Salmonella* spp. strains.

Strains	TEM	SHV	Carba	qnrA
P9	+	–	–	–
P14	+	–	–	–
P15	+	–	–	–
P16	+	–	–	–
P17	–	–	–	–
P19	+	–	–	–
P29	+	–	–	–
P70	–	–	–	–
P362	+	–	–	–
P368	–	–	–	–

The TEM gene was identified in seven out of ten strains, namely P9, P14, P15,

P16, P19, P29, and P362. This high frequency suggests a widespread distribution of this gene among the analyzed isolates. TEM genes are generally associated with the production of β-lactamases, enzymes capable of hydrolyzing certain penicillins and cephalosporins, thereby conferring resistance to β-lactam antibiotics.

The strains P17, P70, and P368 did not present any of the targeted genes, which may indicate either the absence of these specific resistance mechanisms or the presence of other genetic determinants not investigated in this study.

The complete absence of the SHV gene indicates that this type of β-lactamase is not involved in the resistance of the tested strains. Similarly, no Carba genes were detected, suggesting the absence of carbapenemases, which are major enzymes responsible for resistance to carbapenem antibiotics. Finally, the absence of the qnrA gene indicates that no plasmid-mediated quinolone resistance mechanism linked to this gene was identified.

3.5.2. *Staphylococcus aureus* Strains

Molecular analysis of *Staphylococcus aureus* strains revealed the presence of the mecA resistance gene in the clinical strain S6, while it was absent in the reference strain ATCC 25923 (Table 6).

The presence of the mecA gene in strain S6 indicates a resistance mechanism to β-lactam antibiotics, including penicillins and cephalosporins, through the production of an altered penicillin-binding protein (PBP2a). This profile is characteristic of methicillin-resistant *Staphylococcus aureus* (MRSA) strains.

In contrast, the absence of the mecA gene in the ATCC 25923 strain confirms its susceptibility status, as it is used as a reference strain for quality control in microbiological testing.

Table 6. Distribution of resistance genes among *Staphylococcus aureus* strains.

Strains	mecA
S. aureus ATCC 25923	–
S6	+

4. Summary of Resistance Gene Identification Results

Molecular analysis of *Salmonella* spp. strains revealed the presence of the TEM gene in several isolates (P9, P14, P15, P16, P19, P29, P362), while strains P17, P70, and P368 did not carry any of the targeted genes.

Furthermore, none of the SHV, Carba, or qnrA genes were detected in the studied *Salmonella* spp. strains.

Regarding *Staphylococcus aureus* strains, the mecA gene was absent in the reference strain (*S. aureus* ATCC 25923) but present in strain S6, indicating a β-lactam-resistant profile.

4.1. Detection of the TEM β-Lactam Resistance Gene in *Salmonella* spp. Isolates by Agarose Gel Electrophoresis

Figure 1 presents the results of PCR amplification of the TEM gene in *Salmonella*

spp. isolates following agarose gel electrophoresis. The profile includes a molecular weight marker (M), a positive control (PC), a negative control (NC), and the analyzed samples (E). The presence of amplification bands in the wells corresponding to the samples indicates detection of the target TEM gene fragment in the concerned isolates. The positive control confirms the proper functioning of the PCR reaction, whereas the absence of a specific signal in the negative control helps rule out contamination. Overall, the electrophoretic profile is consistent with the presence of the TEM gene in some *Salmonella* spp. isolates, suggesting that these isolates may harbor a genetic determinant associated with *β*-lactam resistance.

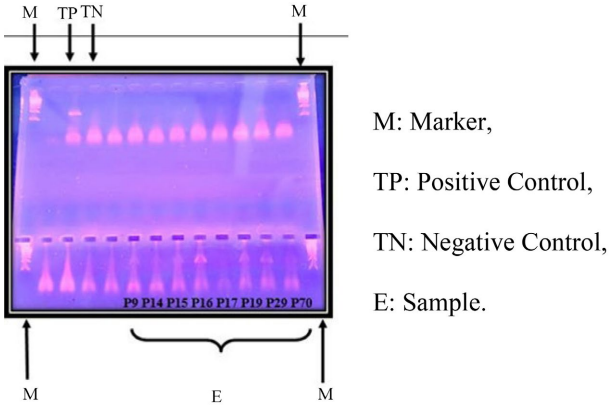


Figure 1. Agarose gel electrophoresis showing amplification of the TEM resistance gene in *Salmonella* spp. isolates.

4.2. Electrophoretic Profile of TEM Gene Amplification in Isolates P362 and P368

Figure 2 presents the electrophoretic migration of PCR products obtained during the detection of the **TEM** gene, particularly in isolates **P362 and P368**. The gel is flanked by molecular weight markers (M), allowing assessment of the position and apparent size of the amplified fragments. The presence of bands at positions corresponding to the samples indicates amplification of the target fragment in the concerned isolates. This profile therefore supports the results obtained for **TEM**

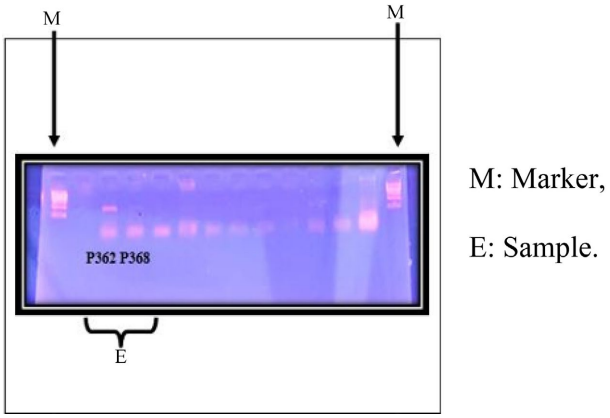


Figure 2. TEM gene migration photograph.

gene detection and suggests that isolates P362 and P368 harbor this genetic resistance determinant, provided that the observed band size corresponds to the theoretical amplicon size.

4.3. Absence of Detectable SHV Gene Amplification in the Isolates Studied

Figure 3 presents the results of SHV gene detection by PCR followed by agarose gel electrophoresis. The profile includes a molecular weight marker (M), a positive control (PC), a negative control (NC), and the analyzed samples (E). No specific amplification product corresponding to the SHV gene is observed in the samples presented, in contrast to the positive control. This result indicates an absence of detectable SHV gene amplification in the isolates studied under the experimental conditions used. Thus, among the resistance determinants investigated, the SHV gene was not detected in the analyzed isolates. However, it is scientifically preferable to refer to the absence of detectable amplification rather than to the absolute absence of the gene.

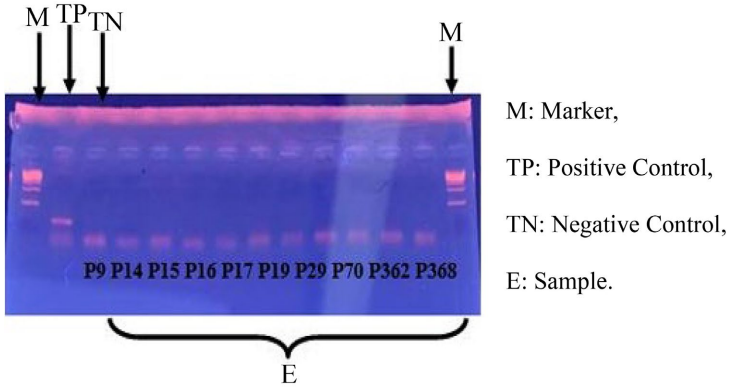


Figure 3. Agarose gel electrophoresis showing absence of SHV gene amplification.

4.4. Electrophoretic Profile of the Detection of Carbapenemase Genes in the Isolates Studied

Figure 4 shows the electrophoretic profile obtained following PCR amplification

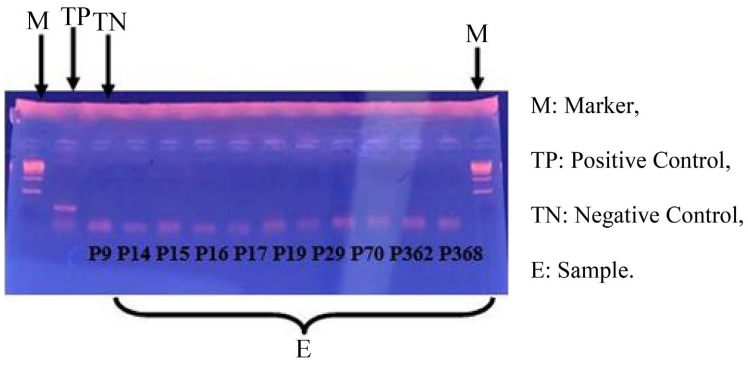


Figure 4. Gel electrophoresis image showing Carba gene migration.

of targets corresponding to carbapenem resistance genes ("Carba"). The gel includes a molecular weight marker (M), a positive control (PC), a negative control (NC), and the samples analyzed (E). The profile allows assessment of the possible presence of amplification fragments corresponding to the target genes. In the profile presented, no specific band clearly identifiable as corresponding to the target amplicon is observed in the samples. This result is therefore consistent with an absence of detectable amplification of the targeted carbapenemase genes in the isolates analyzed.

4.5. Electrophoretic Profile of qnrA Gene Detection in the Isolates Studied

Figure 5 presents the results of qnrA gene amplification by PCR followed by agarose gel electrophoresis. The profile includes a molecular weight marker (M), a positive control (PC), a negative control (NC), and the different samples (E). Examination of the gel does not clearly reveal, in the samples presented, a band corresponding to the expected qnrA gene fragment. This result therefore indicates an absence of detectable qnrA gene amplification in the isolates studied. However, the absence of this specific gene does not exclude the presence of other genetic mechanisms of quinolone resistance that were not investigated in this analysis.

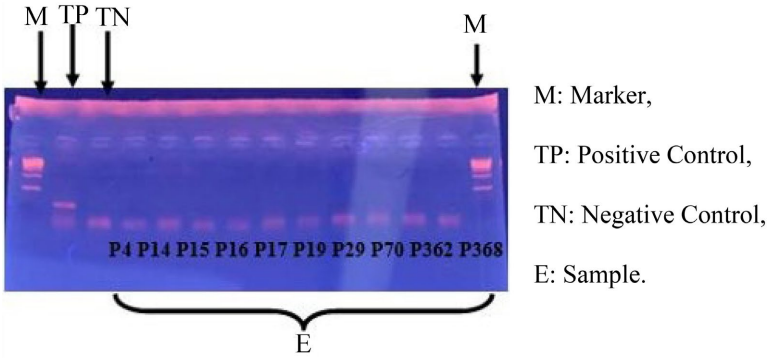


Figure 5. qnrA gene migration photograph.

4.6. Detection of the mecA Gene *in Staphylococcus aureus* Strain S6

Figure 6 presents the result of mecA gene amplification by PCR in *Staphylococcus aureus* strain S6, followed by agarose gel electrophoresis. The profile includes a molecular weight marker (M) and the analyzed sample (E). The presence of a band corresponding to the expected region of the amplification product in strain S6 is consistent with the detection of the mecA gene in this isolate. The detection of this gene is an important finding because it is associated with a major mechanism of β-lactam resistance in *S. aureus*. Nevertheless, the result should be interpreted in relation to the expected amplicon size and the validation of the experimental controls.

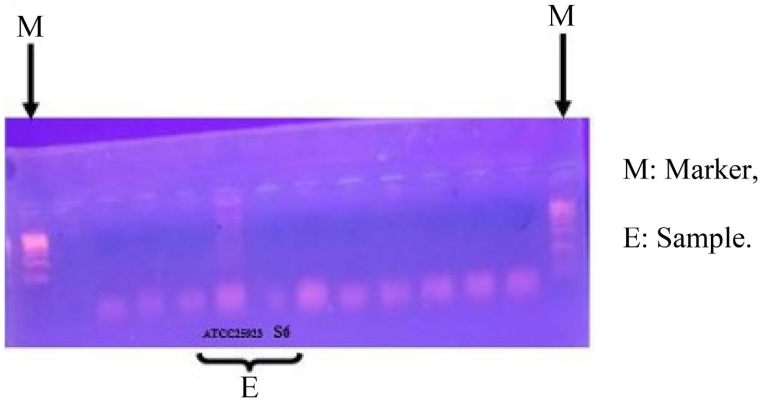


Figure 6. Detection of mecA gene in *Staphylococcus aureus* strain S6.

5. Discussion

The results of the present study highlight the biological potential of *Mucuna pruriens* seeds marketed in Guinea-Conakry through their phytochemical composition, antibacterial activity, acute toxicity profile, and association with antimicrobial resistance determinants.

The extraction yields obtained revealed a clear superiority of the ethanolic extract (42.18%) compared with the aqueous extract (14.52%). This difference may be attributed to the greater ability of ethanol to solubilize a broader range of phytochemicals, including compounds of intermediate polarity such as flavonoids, alkaloids, and terpenoids. Similar observations have been reported by Sultana *et al.* [31] and Dirar *et al.* [32], who emphasized the influence of solvent polarity on extraction efficiency and recovery of bioactive compounds.

Phytochemical screening demonstrated the presence of several secondary metabolites, including alkaloids, flavonoids, saponins, gallic tannins, mucilages, reducing compounds, and steroid-terpenes. These findings support previous reports describing Mucuna species as rich sources of biologically active compounds. According to Lampariello *et al.* [33], the phytochemical diversity of Mucuna contributes significantly to its medicinal value. Flavonoids and polyphenolic compounds have been associated with antioxidant and anti-inflammatory activities [34], whereas alkaloids and saponins are known to possess antimicrobial and immunomodulatory properties [35].

The antibacterial activity of the extracts varied according to the bacterial strains tested. Inhibition zone diameters ranged from 0 to 12 mm, indicating weak to moderate antibacterial activity. The ethanolic extract generally exhibited greater activity than the aqueous extract, particularly against strains P29, P17, P368, S6, and *Staphylococcus aureus* ATCC 25923. These findings suggest that ethanol was more effective in extracting compounds responsible for antibacterial activity. Similar results were reported by Parekh *et al.* [36], who observed stronger antibacterial effects in methanolic extracts compared with aqueous extracts. The absence of activity against strains such as P9, P14, and P19 may be explained by intrinsic or acquired resistance mechanisms, including reduced membrane permeability and

active efflux systems, as described by Coates and Hu [37]. The higher susceptibility observed in *Staphylococcus aureus* compared with several Gram-negative isolates agrees with the observations of Davies [38], who reported that Gram-positive bacteria are often more sensitive to plant-derived antimicrobial compounds.

The acute toxicity assessment revealed no mortality following administration of a single oral dose of 2000 mg/kg body weight for either extract. According to OECD guideline 423, these findings suggest that the LD_{50} is greater than 2000 mg/kg, indicating relatively low acute toxicity. Similar observations were reported by Ugwah-Oguejiofor *et al.* [28], who found no mortality and an LD_{50} greater than 2000 mg/kg for the aqueous extract of the West African medicinal plant *Caralluma dalzielii*; such findings support the continued value of plant-derived natural products as sources of new therapeutic agents [39]. Nevertheless, Fabricant and Farnsworth [40] emphasized that the absence of mortality during acute toxicity testing does not necessarily guarantee complete safety following repeated or long-term exposure.

Hematological analyses showed differential effects depending on the extract administered. The aqueous extract induced only minor modifications in blood parameters, whereas the ethanolic extract produced significant decreases in red blood cell count, hemoglobin concentration, and hematocrit after 14 days of treatment. These findings suggest a possible influence of some bioactive constituents on erythrocyte production, survival, or membrane stability. Similar hematological alterations have been reported by Cowan [41], who observed that extracts rich in polyphenolic compounds may induce moderate but reversible changes in hematological parameters.

Biochemical analyses revealed significant variations in renal and hepatic markers. The aqueous extract induced a significant reduction in urea levels and an increase in ALT activity, whereas the ethanolic extract produced significant increases in creatinine and AST levels. Elevated transaminase activities may indicate mild hepatic stress, while increased creatinine concentrations may suggest an effect on renal function. Comparable biochemical alterations following administration of high doses of medicinal plant extracts have been reported by Hammer *et al.* [42]. However, Rates [43] noted that such variations are often dose-dependent and may remain reversible in the absence of severe histopathological lesions.

An important aspect of the present study was the investigation of antimicrobial resistance genes among the tested bacterial strains. Molecular analysis revealed the presence of the TEM gene in seven *Salmonella* spp. isolates, whereas SHV, Carba, and qnrA genes were not detected. The predominance of TEM-positive isolates suggests a widespread distribution of β-lactam resistance mechanisms among these strains. These findings are consistent with reports from Fair and Tor [44], which highlighted the global dissemination of TEM-type β-lactamases among Enterobacteriaceae. In addition, the mecA gene was detected in the clinical *Staphylococcus aureus* strain S6 but was absent in the reference strain ATCC 25923. The presence of mecA confirms the methicillin-resistant phenotype of strain S6, as previously

described by Turner *et al.* [45].

Interestingly, some bacterial strains carrying resistance genes remained susceptible to the plant extracts, particularly the ethanolic extract. This observation suggests that the antibacterial compounds present in *Mucuna pruriens* may act through mechanisms different from those targeted by conventional antibiotics. Such findings support the growing interest in medicinal plants as potential sources of novel antimicrobial agents capable of overcoming existing resistance mechanisms.

Overall, the results demonstrate that *Mucuna pruriens* seeds possess appreciable phytochemical richness, moderate antibacterial activity against selected multidrug-resistant bacterial strains, and relatively low acute toxicity. Nevertheless, the hematological and biochemical modifications observed, particularly following administration of the ethanolic extract, indicate that additional studies involving chronic toxicity assessment, histopathological analyses, and isolation of active compounds are necessary to fully elucidate the mechanisms of action and establish the safety profile of this medicinal plant.

6. Conclusions

This study demonstrated that *Mucuna pruriens* seeds possess promising biological properties. The ethanolic extract showed a higher extraction yield and greater antibacterial activity than the aqueous extract, with inhibition zones reaching 12 mm against *Staphylococcus aureus* ATCC 25923. Phytochemical screening revealed the presence of several bioactive compounds, including flavonoids, alkaloids, saponins, and tannins, which may contribute to the observed antibacterial effects.

The acute oral toxicity study conducted according to OECD guideline 423 showed no mortality at 2000 mg/kg body weight, suggesting low acute toxicity, although some hematological and biochemical changes were observed. Overall, *Mucuna pruriens* appears to be a promising source of antibacterial compounds. Further studies are needed to isolate its active constituents and assess its long-term safety and therapeutic potential.

Ethics Approval and Consent to Participate

This study was conducted in accordance with internationally accepted ethical guidelines for animal experimentation. All experimental procedures involving Wistar rats were carried out following the recommendations of the OECD guidelines for the testing of chemicals (OECD 423) for acute oral toxicity assessment. Ethical approval was obtained from the institutional scientific committee of the laboratory where the study was conducted. All efforts were made to minimize animal suffering and reduce the number of animals used.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions

Clémentine Michodjehoun and Steven Chokki conceptualized and designed the study, supervised the research activities, and contributed equally to the interpretation of the results and the critical revision of the manuscript. Alphonse Keller Konkon participated in the methodological design and provided scientific guidance on antimicrobial assays and data validation. Floride Bernice T. Abatti contributed to the laboratory experiments, particularly in the phytochemical screening and antibacterial activity evaluation. Rodrigue Azonwakin Akotegnon and Arnaud Noudéhouénou Kohonou were involved in the execution of experimental work, data collection, and preliminary statistical analysis. Halfane Toure Lehmane contributed to the microbiological analyses and handling of multidrug-resistant bacterial strains. Fatoumata Bah participated in sample preparation, data recording, and laboratory support. All authors read, revised, and approved the final version of the manuscript.

Acknowledgements

The author sincerely acknowledges the laboratory staff for their technical assistance during the experimental work. Special thanks are also extended to colleagues for their valuable support and constructive discussions throughout the study.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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