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In vitro Antibiotic Activity of *Mucuna pruriens* Ethanolic Extract

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Abstract: The aim of this study was to elucidate the antibiotic activity of ethanolic extract of *Mucuna pruriens* leaves. The plant leaves were collected from the Institute of Agriculture, Moor Plantation, dried, milled and extracted with ethanol. The ethanolic extract was tested against human pathogens of clinical importance employing the agar well diffusion technique at different concentrations of 100mg/ml, 250mg/ml and 500mg/ml respectively. The zone of inhibition and minimum inhibitory concentration of the extract were determined respectively. Results showed that antibiotic susceptibility testing of the ethanolic extract of *Mucuna pruriens* showed various degrees of inhibition against various microorganisms tested. *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Proteus mirabilis, Serratia marcescens* and *Bacillus cereus* were susceptible to the extract at concentrations 100mg/ml, 250mg/ml and 500mg/ml respectively. *Salmonella typhi, Shigella dysenteriae* and *Clostridium perfringens* were susceptible at concentration 250mg/ml and 500mg/ml respectively while *Aerobacter aerogenes* and *Proteus vulgaricus* were susceptible at concentration 500mg/ml respectively. The plant extract demonstrated broad-spectrum antibiotic effects which may be attributed to the presence of alkaloids and tannins.

Key words: *Mucuna pruriens* leaves · Antibacterial Activity · Ethanolic Extract · Itching Beans · Phytochemicals

INTRODUCTION

The alarming rate of antibiotic resistance by bacteria pathogens in recent years has made treatment of bacterial infections a difficult process posing health problems worldwide. Furthermore, antibiotics have been reported by several authors to interfere with natural gut microflora thereby impairing the immunologic functions of these intestinal microflora [1, 2]. Due to these reasons coupled with recent pandemic attacks, there is a paradigm shift from the conventional orthodox drugs to medicinal plants leveraging on the new bioactive components as well as reduced side effect [3].

Mucuna pruriens, belonging to the family *Fabaceae* is a leguminous plant known as Velvet or Buffalo beans in English. In Nigeria, this plant is called Igenekpe in Ebira, Werepe or Yerepe in Yoruba, Agbala in Igbo, Upupu in Kiswahili and Inyelekpe in Igala [4]. The plant is famous for its extreme itch dermatitis when in contact by humans particularly with the young foliage and the seed pods due to its long stinging hairs. The seed powder has been reported to show faster hypothermic effect, anti-parkinson effect, anti-anxiety effect as well as increased libido effects and neuroprotective effects which may be related to its anti-oxidant property [2, 5].

The leaves of this plant have also been reported to show anti-protozoan, anti-inflammatory, anti-diabetic, hypoglycemic, antivenom as well as antibiotic activities [4, 6]. However, the antimicrobial activity of *Mucuna pruriens* by researchers especially in Nigeria is scanty. Hence, this study aimed to elucidate the *in vitro* antibiotic property of this plant.

MATERIALS AND METHODS

Collection of Plant Materials: Fresh leaves of *Mucuna pruriens* were collected from Palm Plantation Institute of Agricultural Research and Training, Ibadan.

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The plant materials were identified and authenticated with a voucher specimen number: UIH-22563 at the Department of Botany, University of Ibadan. The leaves were thoroughly washed and shade dried at room temperature for 10 to 15 days after which they were milled using a milling machine and stored in air-tight bag until used [2].

Ethanolic Extraction of Plant Materials: About 500g of the dried and milled leaves of *Mucuna pruriens* was weighed and soaked in 2000ml of 95% ethanol in a conical flask, covered with foil paper and protected from sunlight for 48 hours with regular stirring with a stirring rod to ensure proper mixing. The ethanolic constituent was then filtered using Whattman No.1 filter paper after which the filtrate was concentrated using a rotatory evaporator at a temperature of 40°C. The concentrate was stored in a labeled, sterile amber bottle in a refrigerator at 4°C [7].

Bacterial Strains Used: Pure cultures of clinical pathogenic bacterial isolates were obtained from Microbiology Laboratory of Institute of Agricultural Research and Training, Moor Plantation, Ibadan and Department of Microbiology Laboratory, University of Ibadan (UI), Ibadan. They are: *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aureginosa, Bacillus subtilis, Proteus mirabilis, Serratia marcescens, Bacillus cereus, Shigella dysenteriae, Clostridium perfringens, Aerobacter aerogenes* and *Proteus vulgaricus*. They were sub-cultured on nutrient agar and stored in a refrigerator.

Antibiotic Sensitivity Testing: Agar well diffusion method was used as described by [8]. Nutrient agar was prepared according to manufacturer's description, sterilized at 121°C. Then 20 ml was poured in a petri dish at 40°C and allowed to solidify. The agar was inoculated with 2ml of prepared desired inoculum prepared with distilled water using spread plate technique. Wells were made in the agar with a 6mm cork-borer and aliquots of 1ml extract prepared in different concentrations of 100mg/ml, 250mg/ml and 500mg/ml was discharged into each well in triplicate respectively. A disc containing oxytetracycline was used as control for each of the organisms. They were incubated at 37°C for 48 hours.

Determination of Zone of Inhibition and Minimum Inhibitory Concentration (MIC): The zone of inhibition was determined by measuring the diameter of clearance of the extracts and the antibiotic used as control. The mean values were expressed in millimeter; the minimum inhibitory concentration was determined using the lowest value of extract concentration that showed clearance.

Determination of Phytochemicals: The methodology used in phytochemical analysis is in agreement with the protocol carried out by Telal, *et al*. [1].

Test for Alkaloids: A small proportion of the ethanolic extract was acidified with a few drops of Dragendroff's reagent and the appearance of an orange brown precipitate indicates the presence of alkaloid.

Test for Cardiac Glycosides: To a small proportion of the ethanolic extract filtrate, few drops of aqueous NaOH were added followed by a few drops of two (2%) percent 3, 5 – dinitro benzoic acid. The presence of pink coloration indicates the presence of cardiac glycosides.

Test for Flavonoids: To a small proportion of the ethanolic extract filtrate, 5ml of dilute ammonia solution is added which is followed by 1ml of H_2SO_4. A yellow coloration that disappears indicates the presence of glycosides.

Test for Steroids: Two (2) ml acetic anhydride was added to a small proportion of ethanolic extract followed by two (2) ml H_2SO_4. A violet coloration changed to blue indicates the presence of steroid.

Test for Tannins: To a small proportion of the extract, a few drops of ferric chloride were added. A greenish black coloration indicates a positive result for the presence of tannins.

Test for Phlobatamins: To two (2) ml of the ethanolic extract was added dilute Hcl and was observed for a red precipitate which indicates the presence of phlobatamins.

Test for Saponnins: A drop of 5% sodium bicarbonate solution was added to 0.5ml of ethanolic extract which was shaken vigorously and kept for three minutes. The appearance of honeycomb like froth indicates the presence of saponins.

Test for Terpenes: Five (5) ml extract was mixed in two (2) ml of chloroform, three (3) ml concentrated H_2SO_4 was added to form a layer. A reddish brown coloration interface indicated presence of terpenes.

Test for Phenols: Ten (10%) percent ferric chloride solution was added to two (2) ml of the extract in a test tube in drops and a bluish black coloration indicated the presence of Phenol.

Table 1: The quantitative analysis of phytochemical *Mucuna pruriens* leaves

Phytochemicals Tested	Strength of Yield
Alkaloids	+++
Tanins	++
Phlobatamins	+
Saponins	++
Flavonoids	+
Steroids	++
Terpenes	+
Phenol	+++
Cardiac glycoside	++

Key: +++, strong; ++, moderate; +, mild

Table 2: The antimicrobial activity of *Mucuna pruriens* leaves

Zones of Inhibition (mm)					
Concentrations of *Mucuna pruriens* (mg/ml)					
Microorganisms	100	250	500	MIC (mg/ml)	Oxytetracycline (0.03mg/ml)
Pseudomonas aeruginosa	5	9	14	100	11
Escherichia coli	2	5	12	100	7
Staphylococcus aureus	2	4	15	100	10
Bacilus subtilis	3	6	9	100	7
Proteus mirabilis	2	7	10	100	8
Serratia marcescens	3	5	10	100	5
Bacilus cereus	1	2	5	100	7
Salmonella typhi	-	3	10	250	8
Shigella dysenteriae	-	4	11	250	10
Clostridium perfringens	-	2	9	250	5
Aerobacter aerogenes	-	-	6	500	8
Proteus vulgaricus	-	-	6	500	5

RESULTS

The quantitative phytochemical analysis of the plant is showed in Table 1. Results showed strong presence of alkaloids and phenols. Also, moderate presence of tannins, saponins, steroids and cardiac glycosides were observed. Phlobatamins, flavonoids and terpenes were mildly present.

As shown in Table 2, the plant showed dose-dependent (500mg/ml > 250mg/ml > 100mg/ml) antimicrobial activities against all the isolates comparable with that of the standard (control), oxytetracycline. The order of deceasing activities: *S. aureus, P. aeroginosa, E. coli* and *S. dysenteriae.* The least antimicrobial activity was against *A. aerogenes* and *P. vulgaris*.

DISCUSSION

The presence of alkaloids, phenols, flavonoids, saponins, steroids, tanins, glycosides and terpenes (terpenoids) observed in the quantitative phytochemical analysis of ethanolic extract of *Mucuna pruriens* (Table 1) is similar to the findings of Murugan and Mohan [2] and Nebedum, *et al.* [9] and BorhadeShobha [10] and Krishnaveni and Hariharan [11] respectively. However, tannins was not present in the findings of Nebedum, *et al.* [9] while glycoside was absent in the report of Borhade Shobha [10]. The discrepancy between this study and Nebedum, *et al.* [9] may due to differences in the plant parts studied. While this study focused on the ethanolic extraction of *Mucuna pruriens* leaves, Borhade Shobha [10] used seeds of the same plant. Furthermore, saponins and steroids were absent in the findings of Divya, *et al.* [12] and this may be due to the type of solvent which used in the extraction of the plants phytochemical as they used methanol in their own study.

Additionally, the observed quality of alkaloids in this study was slightly higher and can be compared to the findings of Nebedum, *et al.* [9], while alkaloids and phenol in this study was far greater than the report of Borhade Shobha [10]. This may be attributed to the different parts of *Mucuna pruriens* studied. Nonetheless, the quality of saponins, steroids, tannins and glycosides observed in this study agrees with the findings of Nebedum, *et al.* [9] and Borhade Shobha [10] respectively except flavonoids which was slight lesser but comparable to their findings.

The presence of phlobatamins as part of the phytochemical constituents has not been reported from any of the findings above including report from Padma, *et al.* [13] who also studied the ethanolic extraction of *Mucuna pruriens* leaves. However, there is need to further study the presence of phlobatamins in *Mucuna pruriens* plant.

The antibiotic susceptibility testing of the ethanolic extract of *Mucuna prurient s*howed various degrees of inhibition against various microorganisms tested (Table 2). Bacteria species such as *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Proteus mirabilis, Serratia marcescens. Bacillus cereus* was susceptible to the extract at concentration 100mg/ml, 250mg/ml and 500mg/ml respectively. *Salmonella typhi, Shigella dysenteriae* and *Clostridium perfringens* were susceptible at concentration 250mg/ml and 500mg/ml respectively while *Aerobacter aerogenes* and *Proteus vulgaricus* were susceptible at concentration 500mg/ml respectively. This shows a dose-dependent and broad spectrum antibiotic activity. Also, this indicates that different concentrations are required for different types of bacteria species.

The observation that all the bacteria species in this study were susceptible to *Mucuna pruriens* leaves extract at different concentrations is comparable to the works of Abraham, *et al.* [4] and Salau and Odeleye [14]. The former, reported that *Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis* were susceptible to the methanolic leaf extract of *Mucuna pruriens* while the later reported *Pseudomonas aeruginosa, Salmonella typhi* and *Shigella dysenteria* were susceptible to the same plant extract. This agreement may be a validation of the claimed antibiotic properties of the plant including its leaves.

The minimum inhibitory concentration obtained in this study was slightly higher than the report of Salau and Odeleye [14], far greater than the report of Rajeshwar, *et al.* [15] and slightly lesser but can be compared to the report of Abraham*, et al.* [4]. This may be as a result of different organisms used; being susceptible at different concentrations. The antibacterial activity of *Mucuna pruriens* has been attributed to the abundance of alkaloids and tannins. Their microbial action may be related to their ability to inactivate microbial adhesion, enzymes and cell envelope transport proteins as they complex with proteins through hydrogen bonding, covalent bonding as well as their hydrophobic effect. However, all these attributes put tannins in spotlight as a major phytochemical contributing to the antibacterial effect of *Mucuna pruriens* [16].

CONCLUSION

This study has successfully evaluated and elucidated the antibacterial activity of ethanolic extract of *Mucuna pruriens.* The leaves of the plant also contain bioactive components such as alkaloids and tannins which appear to be the drive in its antibacterial activity against human pathogens. However, different concentrations are required for the effective inhibitory effect against different pathogens. The presence of phlobatamins as part of the Phytochemicals needs further study. Further investigation on isolation and purification of chemicals responsible for antibacterial activity as they will serve as novel compounds in the development of drugs and effective treatment of the increasing resistant bacterial infections plaguing humanity.

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