

## Antimicrobial Activities of *Mucuna pruriens* (Agbara) on Some Human Pathogens

Mbajiuka Chinedu Stanley<sup>1</sup>, Obeagu Emmanuel Ifeanyi<sup>2\*</sup>, Ochei Kingsley Chinedum<sup>3</sup>, Ifediora Afoma Chinwe<sup>2</sup>, Okezie Emmanuel<sup>1</sup> and Odo Nkechinyelu Nwamaka<sup>4</sup>

1.Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

2.Diagnostic Laboratory Unit, University Health Services Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

3.Department of H.S.S. and Laboratory, FHI 360 Country Office, Plot 1073-A1 Garki, Abuja, FCT, Nigeria.

4Department of Applied Microbiology, Ebonyi State University, Abakaliki, Nigeria.

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**Abstract:** Increasing rate of development of resistance to commonly used antibiotics have led to search for newer, more effective, affordable and easily available drugs. In this study, the organic and aqueous extracts of *Mucuna pruriens* seeds were obtained using a Soxhlet extractor. The antimicrobial activities of the extracts were tested on some human pathogens namely *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*, using 0.3g/ml concentration and the standard method of agar disc diffusion assay. Results obtained showed that the extract have highest inhibitory effect on the gram positive *S. aureus*, lower effect on the gram negative *E. coli* and no inhibition on the fungi (*C. albicans*). Acetone extract produced the highest zone of inhibition (97mm), followed by ethanol (6mm), and water (4mm) extract respectively. Minimum inhibitory concentration assay of 0.0125 and 0.025g/ml for the organic extracts, and 0.025 and 0.05g/ml for the aqueous extracts on *S. aureus* and *E. coli*, respectively. The study proved that *Mucuna pruriens* seeds, apart from their roles as food additives and supplements, can also be utilized as effective and cheap source of antimicrobial agent.

**Keywords:** Antimicrobial activities of *Mucuna pruriens*, some human pathogens, minimum inhibitory concentration and zone of inhibition.

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### I. Introduction

Medicinal plants have been used for centuries as remedies for human diseases, and offer new sources of biologically active chemical compounds as antimicrobial agents (Das et al., 2010). There has been growing interest in the investigation of the alternative route for the substitution of synthetic chemicals, side effects of which are always in question (Yerra et al., 2005). For this reason, the essential oils and the extracts of many plants have been prepared and screened for their antimicrobial activities, leading to accumulation of large number of reports in the literature concerning these properties (Salau and Odeleye, 2007). Out of several hundred thousand medicinal plant species around the globe, only a small portion has been investigated both phytochemically and pharmacologically (Hostettmann, 1999). It has been estimated that 14-28% of higher plants derived components were discovered after following up on ethno-medical use of plants (Neube et al., 2008).

The side effects and the resistance that pathogenic microorganisms build against antibiotics has been given rise to a growing interest in the investigation of biologically active compounds from plants for the discovery of new antimicrobial agents (Yerra et al., 2005). Resistance to penicillin by *S. aureus* was first reported in 1942, and by 1960, more than 80% of both community and hospital acquired staphylococcal isolates were resistant to penicillin (Lowy, 2003). The development of resistance to the newest antibiotics by the microorganisms causing most of the infectious diseases with debilitating effects made the case worse (Adekunle and Adekunle, 2009).

Recently, the acceptance of traditional medicine as an alternative form of healthcare and the development of microbial resistance to available antibiotics has led the researchers to investigate the antimicrobial activity of medicinal plants (Adekunle and Adekunle, 2009). Another driving factor for the renewed interest in plant antimicrobials in the past 20 years has been the rapid rate of plant species extinction (Lewis et al., 1995), as there is a feeling among natural products-chemists and microbiologists alike that the multitude of potentially useful phytochemical structures which be synthesized chemically is at risk of being lost irretrievably (Borris, 1996).

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious disease while simultaneously mitigating many of the side effects often associated with synthetic antimicrobials (Iwu et al., 1999).

In Kenya about 400 plant species have been recorded to be used in traditional remedies(Kokwaro,1976).In the rural areas,even when western health facilities are available,traditional medicine is viewed as an efficient and acceptable system from a cultural perspective(Munguti,1997) and as a result,traditional medicine usually exists side by side with western forms of healthcare(Sindga,1994).Generally in developing countries,where bacterial infections are prevalent due to inadequate sanitation,poor hygiene and overcrowded conditions.It is estimated that about 80% of the population rely on traditional medicine for their primary health care.There arises a need therefore to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies(Esther and Johannes,2003).

Although there are other approaches to selecting plants species for biological investigation,selecting species that are used in traditional medicine is reported to be more valuable,and many drugs that are of plant origin were discovered from plants used in traditional medicine(Cotton,1996).

#### **AIMS AND OBJECTIVES OF THE STUDY**

The incidence and increasing frequency of microorganisms that are resistant to common and generally accepted effective first choice drugs is on the increase.As this resistivity increases the need for new and/or alternative therapy becomes necessary(Adekunle and Adekunle,2009).This study was designed to assess potentials of the aqueous extracts and organic(ethanol and acetone) extracts of mucuna pruriens seeds on some human pathogens.

- |                |   |
|----------------|---|
| -Gram negative | -Escherichia coli(Enterobacteriaceae)     |
| -Gram positive | -Staphylococcus aureus(staphylococcaceae) |
| -Fungi         | -Candida albicans(Ascomycota)             |

### **II. Materials Method**

#### **SOURCE OF PLANT AND IDENTIFICATION**

The seeds of *Mucuna pruriens* were collected and identified at the National Root Crop Research Institute,Umudike.

#### **SOURCE OF MICROORGANISMS AND IDENTIFICATION**

The microorganisms *S.aureus*,*E.coli* and *C.albicans* were obtained from the stock culture of Federal Medical Centre,Umuahia,Abia State,Nigeria.

#### **PREPARATION OF CRUDE EXTRACTS OF THE PLANT MATERIAL**

Extraction methods involve separation of medicinal active functions of plant tissue from inactive/inert components, by using selective solvents and extraction technology.Solvents diffuse into solid plant tissues and solubilize compounds of similar polarity.Quality of plant extract depends on plant material,choice of solvents and the extraction(Das et al.,2010).

The extraction method that has been widely used by researchers is plant tissue homogenization in solvent(Basri and Fan,2005).

#### **AQUEOUS EXTRACTION**

The seeds were dried in shade,and powdered in a mechanical grinder to a fine paste of uniform consistency(Yerra et al.,2005).

100g of the powder was soaked in 750ml of distilled water and allowed to stand for 24hr(Aguiyi et al.1996).

#### **ORGANIC EXTRACTION**

100g of the powdered seed was soaked in 750ml of ethanol and acetone respectively,for 72hr,and extracted using a Soxhlet extractor at a temperature not exceeding the boiling point of the solvent(Kianbakht and Jahaniani,2003).The extracts were filtered using Whatman filter paper(No.1) and then concentrated in a vacuum and dried(Das et al.,2010).

#### **PREPARATION OF MEDIA,DISC AND MICROORGANISMS**

**Media:**9.5g of Mueller Hinton agar was weighed aseptically and dissolved in 250ml of distilled water(Mueller and Hinton,1941).The solution was sterilized by autoclaving at 121°C/15pa for 15 minutes and allowed to cool to 45-50°C.20ml of the molten agar was poured into petri-dishes and allowed to solidify(Das et al.,2010).

**Discs:**Discs were made from Whatman No 1 filter paper,using a 6mm perforator.The discs were placed in a bottle and sterilized in a hot air oven at 60°C for 10 mins.

**Microorganisms:**Solutions of the microorganisms were made,using peptone water,and standardized to the McFarland's O.D.turbidity standard.This gave a concentration of 10<sup>8</sup>C.F.U/ml of the microorganisms(Das et al.,2010).

### **STERILIZATION AND STERILITY TEST OF MATERIALS**

**Sterilization:**Media were sterilized by autoclaving at 121°C/15pa for 15 minutes, followed by cooling to 45-50°C.

Glass wares such as petri-dishes, pipettes, test tubes, etc were sterilized by dry heat, using the hot air-oven set at 150/160°C for 2 hours.

#### **Sterility test**

**Media:**100ml of agar was prepared, autoclaved and allowed to cool to 45-50°C. 20ml of the molten agar were poured into 3 sterile petri-dishes, allowed to solidify and then incubated at 37°C for 72 hours. No growth on the medium after incubation indicated sterility of both the media and the petri-dishes.

Solutions like distilled water were plated on sterile petri-dishes and incubated at 37°C for 24 hours. No growth indicated sterility.

### **ANTIMICROBIAL SCREENING OF PLANTS EXTRACTS**

Antimicrobial activity was measured using the standard method of agar disc diffusion assay. This method has been widely used to assay plant extract for antimicrobial activity (Eugene et al., 2006).

In this method, 6mm sterilized filter paper discs (Whatman no 1) are saturated with filter sterilized plant extract of desired concentration (Salie et al., 1996). The impregnated discs are then placed on to the surface of a suitable solid agar medium such as Mueller and Hinton (Das et al., 2010), trypton soy agar (Lourens et al., 2004), or Nutrient agar (Doughari, 2006). The medium has been pre-incubated with test organisms standardized to inoculum size  $1 \times 10^8$  C.F.U/ml of bacteria, which is equal to McFarland 0.5 turbidity standard (Baris et al., 2006).

0.1ml of standardized inoculum size of each test organism (924hr old), was spread on agar plate surfaces. Paper discs were impregnated in 0.3g/ml of the plant extract (Yerra et al., 2005). The impregnated discs were placed on the medium, suitably spaced, with a disc of gentamicin as positive control. The plates were then incubated at 5°C for 1 hour to permit good diffusion (Das et al., 2010), and then incubated for 24 hours at 37°C (bacteria) and 48 hours at 25°C (fungi) (Baris et al., 2006). After incubation, diameter of zone of inhibition was measured to the nearest whole millimeter, at the point wherein there is a prominent reduction of 80% growth (Salie et al., 1996).

### **DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC)**

**M.I.C:** This is the lowest concentration of antimicrobials that will inhibit the visible growth of microorganisms after an overnight incubation (Das et al., 2010).

The method employed is the broth macrodilution assay. Here is a set of test tubes with different concentrations of plant extract, with equal volumes are prepared. The tubes are inoculated with test microorganisms of standard concentration ( $1 \times 10^8$  C.F.U/ml) and incubated at 37°C for 24 hours (Laurens et al., 2004).

After incubation, the tubes are examined for changes in turbidity as an indicator of growth. The first tube that appears clear is taken to be the MIC of the extract.

Indicators like tetrazolium salts or resazurin dye (Umeh et al., 2005), or spectrophotometry (Deviene and Raddi, 2002), are used to determine presence of growth.

For spectrophotometric method, the absorbance is usually at 620nm with negative control as blank (Salie et al., 1996). The lowest concentration which gives a zero absorbance. Reading is the MIC of the plants extract (Salie et al., 1996).

**MBC:** This is the lowest concentration of antimicrobials that will kill microorganisms after an overnight incubation.

From the MIC tubes, the tube after the MIC tube contains the minimal bactericidal concentration (Das et al., 2010).

## **III. Results**

### **YIELD OF EXTRACT**

**Extracts** *mucuna pruriens* seeds was extracted with a Soxhlet extractor using different solvents (acetone, ethanol, cold water). Water gave the highest yield, followed by acetone and ethanol respectively, as shown in table 1. The percentage yield is derived by dividing the yield gotten by the initial gram. Initial gram used was 100g of the powdered seeds in 750mls of solvents.

### **ANTIMICROBIAL SENSITIVITY ASSAY**

Antimicrobial sensitivity assay of the different extracts were carried out using the disc diffusion method against the isolates. The result gotten as shown in table 1 indicates that acetone extract produced the highest

inhibition, 7mm followed by ethanol 6mm, and water 4mm extract respectively. Also *S. aureus*, showed greater sensitivity followed by *E. coli*, *C. albicans* showed no significant sensitivity to the extracts.

**MINIMUM INHIBITORY CONCENTRATION**

M.I.C. of the organic and aqueous extracts were determined using the broth macrodilution assay method. Different concentrations (0.1, 0.05, 0.025, 0.0125, 0.00625, and 0.00313g/ml) of the extract was tested against the different isolates. The results as shown in table v and vi indicates that the M.I.C. of the organic extracts was obtained at a concentration of 0.0125 and 0.025g/ml for *S. aureus* and *E. coli* respectively. While that of the aqueous was at 0.025 and 0.05g/ml for *S. aureus* and *E. coli* respectively.

**Table iii: percentage yield of crude extracts of mucuna pruriens seeds**

Solvent	Weight of yield	% yield
Cold water	17.7g	17.7%
Acetone	17.4g	17.4%
Ethanol	7.8g	7.8%

**Table iv: Mean zone of inhibition (mm) of crude extract using the disc diffusion method**

Solvent	<i>C. albican</i>	<i>S. aureus</i>	<i>E. coli</i>
Gentamycin	7	9	8
Acetone	-	7	6
Ethanol	-	6	6
Water	-	5	4

**Table v: M.I.C. of organic extracts of Mucuna pruriens seeds for different isolates**

Organism	Concentration (g/ml)						MIC
	0.1	0.05	0.025	0.0125	0.00625	0.00313	
<i>E. coli</i>	+	+	+	-	-	-	0.025
<i>S. aureus</i>	+	+	+	+	-	-	0.0125
<i>C. albicans</i>	-	-	-	-	-	-	-

+ = Inhibition  
 - = No inhibition

**Table vi : M.I.C. of extract of Mucuna pruriens seeds for the different isolates**

Organism	Concentration (g/ml)						MIC
	0.1	0.05	0.025	0.0125	0.00625	0.00313	
<i>E. coli</i>	+	+	-	-	-	-	0.05
<i>S. aureus</i>	+	+	+	+	-	-	0.025
<i>C. albicans</i>	-	-	-	-	-	-	-

+ = Inhibition  
 - = No inhibition

**IV. Discussion**

The phytochemical analysis of *Mucuna pruriens* has been reported to contain alkaloids, flavonoids, 5-hydroxytryptamine, saponins, etc. the principal constituents are L-Dihydroxyphenylamine (L-DOPA) and the bioactive alkaloids mucunine, mucunadine, prurienine, and nicotine as well as beta sitosterol, lecithin, oils, gallic acids, etc (Yerra et al., 2005).

In this study, aqueous and organic extracts of seeds of *Mucuna pruriens* were tested on the hospital isolates *E. coli*, *S. aureus* and *C. albicans*. Results of the disc diffusion tests in table iv showed that acetone extract was more effective followed by ethanol and water extracts respectively. Also from the result, *S. aureus* showed greatest sensitivity to acetone, ethanol, and water in that order. *E. coli* showed lower sensitivity to the different extracts, while *C. albicans* showed no significant sensitivity to the extract. Previous report by Salau and Odeleye (2007) on antimicrobial activity of *Mucuna pruriens* leaf against *S. aureus*, *C. albicans* etc indicated that the extract had activity on all tested microorganisms except *C. albicans*. A report by Yerra et al (2005) on antimicrobial activity of *Mucuna pruriens* seeds against *S. aureus*, *E. coli*, *V. cholera*, *B. cereus*, etc indicated positive action against all tested organisms with minimal action on *S. aureus* and none on *V. cholera*. Despite the similarities, the disparity in these reports compared to the findings in this study, may depend on the different materials and methods employed.

Major plant pathogens to the gram negative bacteria(George et al.,2002),which makes the low activity of plant antimicrobials against this group of microorganisms puzzling.The results showed that *Mucuna pruriens* extracts have greater activity on Gram positive bacteria *S.aureus*, than on Gram negative bacteria *e.coli*,and no fungi *C.albicans*.Since most plant pathogens belong to the Gram negative bacteria,this shows that plants might have developed means of delivering their antimicrobials into bacterial cells.Both fungi and Gram negative bacteria have evolved permeability barriers(an outer membrane in Gram negative bacteria,and ergosterol in fungi),(George et al.,2002).By contrast,the single membrane of the Gram positive bacteria is considerably more accessible to permeation by amphipathic toxins.

The fact that extraction agents,acetone,ethanol,and water,produced no visible sign of any activity against the hospital isolates means that the extraction agents made no contributions to the antimicrobial potency of the plant extracts.Recently,Eloff(1998) examined a variety of extractants for their ability to solubilize antimicrobials from plants,rate of extraction,ease of removal,toxicity in bioassay,and acetone received the highest rating.This study on *Mucuna pruriens* has further established these facts.

Nutritional analysis reveal that *Mucuna pruriens* contains crude protein ranged from 20-29%,crude lipid 6-7% total dietary fibre 8-10%,ash 3% and carbohydrates 50-60%.Thus apart from its use as a food supplement,this study has proved that *Mucuna pruriens* can be used as an antimicrobial agent in the treatment of bacterial infections.Its ready availability locally and at cheap affordable prices will surely enhance their application among other uses,as an alternative to antibiotics for effective treatment of microbial infections.

## V. Conclusion

Extracts of seeds of *Mucuna pruriens* have shown antimicrobial effects on some human pathogens.Also,despite the fact that most plant pathogens belong to the Gram negative bacteria,the extract proved more effective against Gram positive bacteria.However,this is a preliminary work and more works are needed to actually determine the active ingredients in this plant extracts.This can help in improving management of the different infectious diseases that are developing resistance to commonly used antibiotics.Furthermore,toxicological studies can also be carried out to determine the reliance on this herb without many side effects.

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