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*Full Length Research Paper*

# Antimicrobial Property of Crude Ethanolic Extract of *Mucuna pruriens* leaves on *Pseudomonas aeruginosa, Salmonella typhi* and *Shigella dysentriae*

**[*]Abraham, O.J.[1], Nwobodo, A.H.[2], Ngwu, B.A.F.[3], Onwuatuegwu, J. T.C.[4], Egbunu, Z.K.[1], Yahaya, O.[1], Amodu, A.E.[1], Onuh, I.[5] and Salihu, A. M.[1].**

[1]Department of Science Laboratory Technology, Federal Polytechnic, Idah, Kogi State, Nigeria.
[2]Department of Microbiology, Enugu State University of Technology Teaching Hospital, Enugu, Nigeria.
[3]Department of Microbiology and Pathology, Ebonyi State University, Abakaliki, Nigeria.
[4]Department of Microbiology, Tansian University, Umunya, Anambra State, Nigeria.
[5]Department of Botany, Federal University, Lafia, Nassarawa State, Nigeria.

Abstract

***Mucuna pruriens* also called velvet bean belongs to the family fabaceae. It is used in traditional medicine to cure worms infestation, dysentery, diarrhoea, snake bite, sexual debility, cough, tuberculosis and diabetes. This research was carried out to determine the antimicrobial activity of crude ethanolic extract of *Mucuna pruriens* leaves on *Pseudomonas aeruginosa*, *Salmonella typhi,* and *Shigella dysenteriae*. Agar well diffusion method was used to study the effect of the plant on the organisms at 300mg/ml, 400mg/ml and 500mg/ml. The Minimum Inhibitory Concentration (MIC) on each organism was 300mg/ml. At the concentration of 300mg/ml the mean zone of inhibition was 10mm, 13mm, and 13mm for *Pseudomonas aeruginosa*, *Salmonella typhi,* and *Shigella dysenteriae* respectively. At 400mg/ml, the mean ZI were 11mm, 18mm and 14 respectively, while at 500mg/ml, the mean ZI were 15mm, 20mm and 20mm on the organisms respectively. The zone of inhibition increased with concentration used. The result obtained showed that this plant could be further studied as alternative means to cure the ailment caused by the aforementioned microorganisms. Therefore, further studies have to be done using other solvents extraction.**

**Keywords**: *Mucuna pruriens*, Antimicrobial activity, Crude ethanolic extract, Agar well diffusion, Minimum Inhibitory Concentration, Zone of inhibition.

## INTRODUCTION

Resistance development of microorganisms to antibiotics is increasing in recent time, thus leading to attention being given to extracts and biologically active compounds isolated from plant species used in herbal medicine (Sundaram *et al.,* 2012). Plants have vast untapped source of medicines requiring further exploration. They are effective in the treatment of infectious diseases while simultaneously devoid of the many side effects often associated with synthetic antimicrobials (Rajeshwar *et al.,* 2005; Malaya *et al.*, 2005).

*Mucuna pruriens* is a leguminous plant known as Velvet or Buffalo beans in English, Igenekpe in Ebira, Werepe or Yerepe in Yoruba, Agbala in Igbo, Upupu in Kiswahili (Alo *et al.,* 2012) and Inyelekpe in Igala. The plant is notorious for extreme itchiness it produces on contact particularly with the young foliage and the seed pods. It can be found in tropical Africa, India and Caribbean (Kumar *et al.,* 2005).

The plant is used in the management of Parkinson's disease due to the L-Dopa content (Gurumoorthi *et al.,* 2012). It also increases testosterone level.

*Corresponding Author Email: josephoyiguh@yahoo.com;
Tel : 2348062908906

***Pseudomonas aeruginosa***

It is a rod shape, gram- negative bacillus actively motile by a polar flagellum. *P. aeruginosa* causes infections like respiratory infections, bacteraemia and septicaemia, central nervous system infections i.e. meningitis and brain abscesses, ear and eye infections, bone and joints infections, urinary tract infections, gastro- intestinal infection, skin and soft tissue infections (Balloy *et al.,* 2007). It can be treated with Ceftazidine, gentamicine, ciprofloxacin etc.

***Salmonella typhi***

*S. typhi* is aerobic to facultative anaerobes. It is a gram negative bacilli that parasitizes the intestines of vertebrate species. This organism affects man through ingestion of contaminated water or food. Salmonella infection can be treated with Chloramphenicol, Ciprofloxacin and Pefloxacin (Cheesbrough, 2000).

***Shigella dysenteriae***

*S. dysenteriae* is a non-motile, non-flagellate, non-sporing, non-encapsulated, gram-negative bacilli. The organism causes bacillary dysentery. This facultative anaerobe is virulent and can grow on ordinary laboratory media (Arora and Arora, 2008).

The presence of fake and adulterated drugs has made it difficult to find original drugs that can perfectly cure infectious diseases in Nigeria leading to resistance development by the organisms. Bacterial resistance to currently available antibiotics has rapidly emerged to a global problem and posing a growing public health risk, further more new antibiotics against Gram negative bacteria appears to be scarce in the market (Rakholiya *et al.,* 2015) and the extract of *Mucuna pruriens* has proven to be a potential source of natural antimicrobial agent. In Nigeria, original drugs cost very highly and this makes it unaffordable to most patients, thereby leading to diseases been prolonged which results to death, thus there is need to research into local alternative and affordable treatment for diseases. Also, a promising trend is the combination of standard antibiotics and natural extracts which enhances activity as well as reduces the amount of drug concentration that kills pathogens (Rakholiya and Chanda, 2012). This present study determined the antimicrobial effect of ethanolic extract of *Mucuna pruriens* on some selected bacteria.

## MATERIAL AND METHOD

### Plant collection and preparation

*Mucuna Pruriens* leaves were collected from their natural habitat in Okenya village in Igalamela /Odolu L.G.A. Kogi State, Nigeria and identified by a plant taxonomist. The leaves were air dried at room temperature and ground into fine powder using a clean mortar and pestle.

### Extraction and preparation of leaf extract

Extraction was carried out using Soxhlet extractor and Ethanol. Hundred gram (100g) of the powdered sample was wrapped in Whattman number 24 filter paper and put in the timble of the extractor and extraction carried out. The extract obtained was dried using a rotary evaporator. Crude extract obtained was wrapped in a black polythene bag and stored in the cupboard until used for the assay.

### Source of bacteria strains

A pure culture of clinical isolates of *Pseudomonas aeruginosa*, *Shigella dysenteriae*, and *Salmonella typhi* were obtained from the Bacteria Research Department, National Veterinary Research Institute Vom, Plateau State. The Isolates were re-confirmed using biochemical tests such as indole, methyl red, Voges-Proskauer test, oxidase, catalase and urease test, $H_2S$ production using Kigler iron agar, Tripple sugar iron agar and Lysine iron agar. Sugar fermentation tests were also carried out.

### Preparation of media

Mueller Hinton agar was used for the sensitivity assay and was prepared according to the manufacturer's manual. Briefly, about 25ml of the media after boiling and autoclaving was poured into each of 90mm diameter sterile Petri dishes to a depth of 4mm. The plates were dried with lids slightly raised at 35-37$^{o}$C in the incubator for about 30minutes.

### Preparation of inoculum

Bacterial inoculum equivalent to 0.5McFarland Barium Sulphate turbidity standard was prepared and used to inoculate Mueller-Hinton agar by spread plating.

### Antimicrobial sensitivity test

The organisms were inoculated onto media by spread plating with 2ml of the organism. Six millimetre (6mm) wells were bored on the medium using a cork borer and filled with appropriate concentration of the plant extract using injection syringe. The plates were allowed to stand for 2 hours to allow diffusion of the extract into the

medium and incubated at 34$^{o}$C and Zones of inhibition were measured after 24hours. The zones of inhibition were compared with that produced by the control i.e. Ciprofloxacin. The assay was carried out in triplicate and the mean value of zones of inhibition and standard error calculated.

### Statistical analysis

Results of zones of inhibition were expressed as mean value ± standard error of the mean (SEM).

## RESULT

### Zone of Inhibition (Mm) Produced by Ethanolic Extract of *Mucuna pruriens* leaves on *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella dysenteriae*.

From the table 1, the zone of inhibition produced at the concentration of 300mg/ml was 10mm *Pseudomonas aeruginosa*, while 400mg/ml produce the zone of inhibition of 11mm and 500mg/ml produced the zone of inhibition of 15mm. The control produced zone of inhibition of 30mm. The Minimum Inhibitory Concentration (MIC) in this case was 300mg/ml.

At the concentration of 300mg/ml, the leave extract produced the zone of inhibition of 13mm *Salmonella typhi*. At 400mg/ml, the zone of inhibition produced was 18mm and at 500mg/ml, it produced the zone of inhibition of 20mm. The control produced zone of inhibition of 30mm. The MIC was 300mg/ml.

At the concentration of 300mg/ml, the extract produced the zone of inhibition of 13mm *Shigella dysenteriae*, while 400mg/ml produced the mean zone of inhibition of 14mm and 500mg/ml produced inhibition zone of 20mm. The control produced the zone of inhibition of 29mm. The Minimum Inhibitory Concentration (MIC) was 300mg/ml.

## DISCUSSION

The quest to develop new broad-spectrum antibiotics by Researchers for treating infectious diseases caused by pathogenic microorganisms has spanned many decades. Prolonged usage of currently available broad-spectrum antibiotics has led to the emergence of drug resistant strains of the pathogens, thus giving rise to demands for novel antimicrobial agents from totally different sources (Rakholiya *et al.,* 2015). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, terpenoids, flavonoids, glycosides quassinoids xanthoxyline etc, some of which have been found to have antimicrobial property invitro and they may serve as alternative, effective, cheap and safe antimicrobials for the treatment of microbial infections. The ethanolic extract of *Mucuna pruriens* leaves had activity on *Pseudomonas aeruginosa* which produce 10mm zone of inhibition at concentration of 300mg/ml. This result disagreed with that of Salau and Odeleye (2007) who obtained zones of inhibition of 30mm, 15mm and 11mm on *Pseudomonas aeruginosa* using concentrations of 240, 160 and 80mg/ml respectively.

The Minimum Inhibitory Concentration (MIC) in this study was 300mg/ml against *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella dysenteriae*. The MIC obtained for this study was higher than 80mg/ml obtained by Salau and Odeleye (2007) for methanolic extract of *Mucuna pruriens* leaves. Although in this study ethanolic extract was used as against methanolic extract used by Salau and Odeleye (2007). Methanol extracts out more phytochemicals than ethanol (Cowan, 1999).

The ethanolic extract of *Mucuna pruriens* displayed activity against *Salmonella typhi* with MIC of 300mg/ml and zone Inhibition of 13mm. These observations are at variance with the result of Ashok *et al.* (2007) who obtained the zone of inhibition of 26mm at an MIC of 100mg/ml using the same ethanolic extract of *Mucuna pruriens* leaves. This difference in MIC and zone of inhibition may be due to differences in strains of organisms used.

The ethanolic extract of *Mucuna pruriens* also had activity on *Shigella dysenteriae* which produce 13mm zone of inhibition at MIC of 300mg/ml. This result does not conform with that of Dhatwalia *et al.* (2009) whose zone of inhibition was 24mm at MIC of 100mg/ml.

Bala *et al*. (2011) reported moderate sensitivity of *Salmonella typhi*, *Shigella sonnei* and *Shigella flexneri* to ethanolic extract of *Mucuna pruriens* leaves. In this study, the extract was observed to have moderate activity on *Shigella dysenteriae* and *Pseudomonas aeruginosa* as well.

The variation in results can be traced to the different part of the plant used. In the study carried out by Dhatwalia *et al.* (2009), the seed of *Mucuna pruriens* was used as against the leaves used in the present study. Other reasons may be the age of plant, time of harvest and variation in quality and quantity of the active constituents which show variation from season to season (Gideon *et al.,* 2012).

Comparatively, *Salmonella typhi* displayed more sensitivity to the extract followed by *Shigella dysenteriae.* This implies that the plant can be administered even locally against diseases caused by these agents especially typhoid fever and dysentery. In a study on extract of Jalapeño pepper slurry by Bacon *et al.* (2016), no inhibition of the organisms was observed due to pH of the of the plant extract.

Plant extracts have been studied against bacteria and fungi for years, but in a more intensified way in the last three decades. During this period, a lot of antimicrobial screening evaluations have been published based on the traditional use of Chinese, African and

**Table 1.** Mean Zones of Inhibition (Mm) of Ethanolic Extract of *Mucuna pruriens* leaves on *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella dysenteriae*

| Organisms | Conc (mg/ml)/Zone of Inhibition (mm) | | | Overall std error | Control(Cipro) 500 | MIC |
|---|---|---|---|---|---|---|
| | 300 | 400 | 500 | | | |
| *Pseudomonas aeruginosa* | 10± 0.272 | 11±0.471 | 15± 0.471 | 0.728 | 30±0.272 | 300 |
| *Salmonella typhi* | 13±0.471 | 18±0.272 | 20±0.720 | 1.054 | 30±0.047 | 300 |
| *Shigella dysenteria* | 13±0.125 | 14±0.243 | 20±0.943 | 1.041 | 29±0.249 | 300 |

Asian plant drugs (Toroglu *et al.*, 2013). The antimicrobial effects of plants are mostly due to the essential oils present in their composition. Compounds such as phenols, flavonoids, aldehydes, ketones, saponins and alcohols are responsible for the antimicrobial activity (Sindhu and Manorama, 2012).

## CONCLUSION

The ethanolic extract of *Mucuna pruriens* leaves was found to have antimicrobial activity at the concentration of 300, 400 and 500mg/ml. Therefore, ethanolic extract of *Mucuna pruriens* leaves can be used as an alternative for the cure of the ailments caused by the study organisms. It can also be possible candidate for the production of modern drugs for the diseases also. Further studies to isolate the active components responsible for the antimicrobial activity should be carried out.

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