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In vitro evaluation of the antibacterial activity of alcoholic extract from *Mucuna pruriens* seed

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Abstract
The present investigation is aimed to find out the efficacy of *Mucuna pruriens* supplemented feeds on the management of vibriosis in lab condition. The antimicrobial activity of alcoholic extract of *Mucuna pruriens* (AEMP) seeds was determined by disc diffusion method with various gram positive and gram negative bacteria. The strongest antibacterial effect was observed in the methanol extract of *Mucuna pruriens* seeds against *Vibrio harveyi* (16.8 mm inhibition zone) and *Vibrio cholera* (9.4 mm inhibition zone) followed by *Escherichia coli* (4.1 mm inhibition zone) and *Staphylococcus aureus* (3.4 mm inhibition zone). Broth dilution method was used to estimate Minimum inhibition concentration (MIC) against selective pathogens. The results suggest that the methanol extract of *Mucuna pruriens* seed phytochemicals has an antibacterial effect may be used along with feed to manage the Vibriosis.

Keywords: *Mucuna pruriens* seed, Antibacterial activity, Disc diffusion method, MIC.

1. Introduction
Vibriosis is a bacterial disease responsible for the mortality of cultured shrimp worldwide. Numerous studies have attempted to control vibriosis by antibacterial synthetic drugs administered directly into the water or via medicated feeds. The presences of antibiotic residues in aquaculture products for human consumption constitute an important threat to public health. Therefore, to make the aquaculture industry more sustainable, new strategies to control infections urgently need. *Mucuna pruriens* is a popular Indian medicinal plant, widely utilized in the multiple pharmacological uses due to the presence of medicinally active phytochemicals. The velvet bean *M. pruriens* is widespread in tropical and sub-tropical regions of the world [1]. It had been evaluated and concluded as a potent medicinal herb in terms of anti cholesterolemic, antiparkinson, antidiabetic, aphrodisiac and antimicrobial [2]. The development of bacterial resistance to presently available antibiotics due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the production cost of synthetic drugs is high and they produce adverse effects compared to plant derived drugs [3]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [4]. Ethanol and methanol solvent extracts of *Mucuna* seed seemed to be brought better extraction of phytochemical compounds [5]. Hence the present study is aimed to investigate the antimicrobial potency of AEMP seed extract against selective multi drug resistant pathogenic bacteria.

2. Materials and Methods
2.1. Plant Extract
The seeds of *M. pruriens* were collected from the local Ayurvedic shop in Pondicherry and the identity was confirmed by traditional and expects of plant biologist. Shade dried seeds were pulverized into fine powder using electric blender. Finely powdered *M. pruriens* seeds were subjected to alcoholic extraction with methanol and ethanol (Analytical grade) (Merck, Inc). The concentrated *M. pruriens* seed extract was dried and stored at 4 ^{o}C until use and this dry extract was subjected for antibacterial assay.

2.2. Bacterial strain used

Microorganism (*Staphylococcus aureus MTCC.* 9542, *Salmonella typhi MTCC.3224, Salmonella paratyphi MTCC 3220, Klebsiella oxytoca MTCC 3030, Klebsiella pneumoniae MTCC 7407, Pseudomonas aeruginosa MTCC 6458, Escherichia coli MTCC1698, Proteus mirabilis MTCC 9493, Vibrio cholerae MTCC 3906, Vibrio harveyi MTCC 7954*) were obtained from the department of Medical Microbiology (Raja Muthiah Medical College hospital), Annamalai University, Annamalai Nagar.

2.3. Determination of Antibacterial activity

The spectrum of antibacterial activity was studied using as test agent a range of ten different pathogenic stains of both gram positive and gram-negative bacteria of which there were one antibiotic agent Erythromycin (C). *In vitro* antibacterial assay was performed by the disc diffusion technique [6]. All bacterial strains were grown in Mueller Hinton Broth medium (Merck) for 24 h at 37 °C. The concentration of bacterial suspensions was adjusted to 10^8 colony forming units (10^8 cfu/ml) in Mueller Hinton Agar. 1 mm discs impregnated in ethanol and methanol extracts of *M. Pruriens* and positive control contained of a standard antibiotic disc were subjected for antibiogram assay. Erythromycin was used as standard antibiogram. After incubation at room temperature (37 °C) for 24 hrs antibacterial activities were expressed in terms of the diameter of zone of inhibition which was measured in mm using vernier caliper and recorded.

2.4. Minimum Inhibitory Concentration

A serial micro-dilution assay was used to quantify the minimum inhibitory concentration (MIC) values of *M. Pruriens* seed methanolic extract against the selective bacterial pathogen which exhibited maximum zone of inhibition in antibacterial assay. The MIC was assessed using tetrazolium violet reduction as an indicator of growth [7, 8]. MIC of plant extract was determined by the method of broth dilution. Lysogeny broth (LB) was used to prepare bacterial aliquots. Aliquots of 10 ml of LB media was placed into 96 well microtitre plates before the plant extract solution was added to the tubes to the final concentration of 100 mg ml^{-1}. Next, the concentration of the plant extract was adjusted ranging from 10-250 µg ml^{-1} by adding different volumes of the LB media. All above samples were autoclaved at 121 ^{0}C and cooling before the test bacteria suspension was added into to the inoculum size of 10^6 CFU ml^{-1} and then incubated at 37 ^{0}C for 24 h. Another diluted solution without adding any bacterium was prepared as control samples. The turbidity of all samples was determined at 540 nm in an ELISA reader (Model: Cybershop microplate reader). The determination was based on the same turbidity of the two subjects of the same concentration, i.e., the highest dilution or least concentration of the extracts that inhibit growth of organism is defined as MIC.

2.5. Statistical analyses

All the data's are expressed as mean ± S.D. Statistical analysis was performed with one-way ANOVA followed by Duncans t-test for multiple comparisons. $P < 0.05$ was considered significant.

3. Results

M. Pruriens methanolic extract exhibited the significant antibacterial effect against series of bacterial pathogens (Figure 1). Among the various strains maximum antibacterial effect was recorded against *Vibrio harveyi* with 16.8 mm diameter of zone of inhibition was recorded against methanol extract of *M. Pruriens. V. cholera* recorded its second far most antibacterial sensitiveness to *M. Pruriens* with 9.4mm followed by *E. coli* with 4.1 mm, *S. aureus* bacteria with 3.4 mm. The least antibiogram was recorded against *S. typhi, K. pneumonia* and *P. aeruginosa*. The control agent erythromycin reflected its antibiotic effect against major tested pathogens.

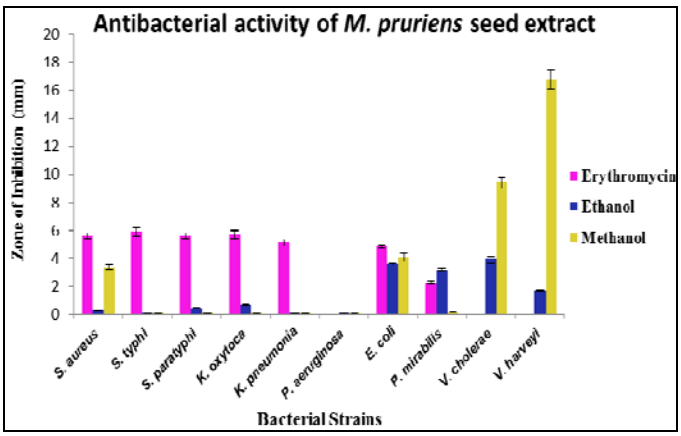

Fig 1: Antibacterial activity of *M. pruriens* seed extract

In the MIC assay *M. pruriens* methanol extract was screened against sensitive bacterial pathogens of *E. coli*, *S. aureus*, *V. cholerae*, and *V. harveyi* bacteria which exhibited maximum antibacterial effect in in-vitro assay (Figure 2). *M. pruriens* extract of 10 µg - 250 µg was screened against four bacteria in using microtitre plate and the bacterial growth inhibition was assessed using optical density measurement at 540 nm. The results are promising enough that extract exhibits the maximum activity of MIC with the range of 25 µg/ml concentration against *V. harveyi,* followed by *V. cholera* at 75-80 µg/ml, *S. aureus* and *E. coli* on 175 µg/ml.

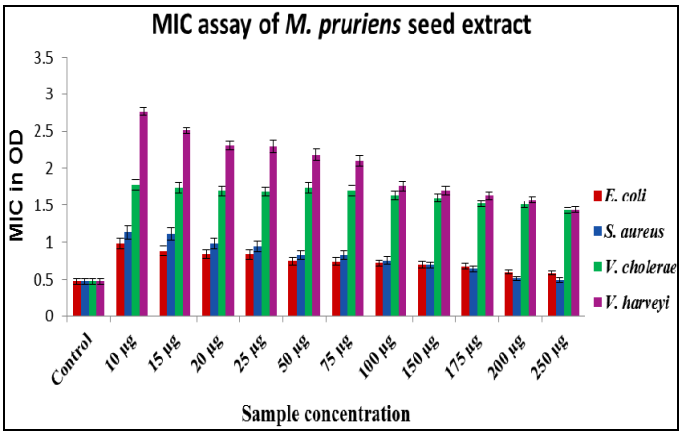

Fig 1: MIC assay of *M. pruriens* seed extract against selective pathogen

4. Discussion

In the present study alcoholic extract of *M. pruriens* seed extract in ethanol and methanol was screened for its antibacterial effect against multi drug resistant human bacterial pathogens. Out of these two extracts methanol extract exhibited a significant antibacterial effect against *V. harveyi* bacteria and *Vibrio cholera*. Rajeshawar *et al.*, [9] reported that the methanol extract of *Mucuna pruriens* had significant *in*

vitro lipid peroxidation and antimicrobial activity. The antibiotic sensitiveness of *M. pruriens* may be due to the immense phytochemicals present in the form of alkaloids and terpenoids. It is reported that *M. pruriens* consists of gallic acid and Bufotenine as its major phytoconstituents [10]. These two alkaloids and tannin derivative is a very good antioxidant and antimicrobial in nature. Tannic acid, which is a mixture of Gallic acid esters of glucose can be used as a topical preparation for cold sores [11]. Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been assigned to tannins. One of their molecular actions is too complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation [12]. Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, and cell envelope transport proteins, they also complex with polysaccharide [13]. This probably explains the reason as to why the plants containing these tannins showed good antibacterial activity.

Stanley *et al.,* [14] has proved that *Mucuna pruriens* seed was effective antimicrobial agent to treat bacterial infection, apart from their roles as food addictives and supplements. It can also be utilized as effective and cheap source of antimicrobial agent. Pujari and Gandhi [15] were corroborated that *Mucuna pruriens* methanol extract were found to be more effective in inhibiting the pathogens compared to ethanol and acetone extracts.

The study showed that *M. pruriens* methanolic seed extract exhibited the significant antibacterial effect against the four bacterial species; *V. harveyi, V. cholera, E. coli* and *S. aureus.* The active compounds in these plants should be traced out which could find place in the treatment of various bacterial infections where they can be used as alternatives to conventional antibacterial drugs. This could reduce the unnecessary use of antibiotics, which is making disease-causing bacteria more resistant to the drugs and diminishing the drug's power to treat life threatening disease in humans and animals.

5. Conclusion

The study has been able to establish and document the importance of *M. pruriens* seed extract in the management of bacterial diseases. The seed extract shows broad spectrum of antibacterial activity against pathogenic bacteria. This indicates that *M. pruriens* seed extract has capability to solve some of the problems caused by bacteria and also this seed extract is used to treat a number of bacterial diseases. The results suggest that extracts of *Mucuna pruriens* seed phytochemicals have an antibacterial effect may be used along with feed to manage the vibriosis. However, further investigation is required to isolate the active compounds responsible for antibacterial activity. These will serve as lead compounds in the manufacture of novel drugs which can use as one of the approaches in the prevention and control of diseases.

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