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Evaluation of Antimicrobial Activity of Ethanolic Extract of Vigna Aconitifolia

Tirupathi Rao Annavarapu*
Aditya Pharmacy College, ADB Road, Surampalem, East Godavari District

ABSTRACT

The rapid spread of multidrug-resistance against conventional antibiotics is a global threat that necessitates the search for alternative therapies from natural sources. In this investigation, the antibacterial potentials of ethanol extracts of moth beam (*Vigna aconitifolia*) extracts were evaluated against *Bacillus subtilis, Staphylococcus aureus, Staphylococcus werneri, Pseudomonas aeruginosa and Escherichia coli, Proteus mirabilis, Kleibsella pneumonia, Pseudomonas putida,* using Agar cup-plate method. The extract showed antimicrobial activities against all the tested gram positive and gram negative bacteria. Ethanol extracts in general exhibited greater antibacterial activity and results were comparable to the standard. Our finding identifies the potential use of moth bean as a natural source of antibacterial agent. Further studies are warranted to elucidate the mechanism of action of the extracts and to identify the active components responsible for the antibacterial activity.

Keywords: Vigna aconitifolia, Antimicrobial activity, Agar cup plate method.

*Corresponding Author Email: tirupathionline@gmail.com
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INTRODUCTION

The unmethodical and indiscriminate use of commercial antimicrobial drugs has lead to the development of multidrug resistance [1, 14], complicating the choice of empirical therapy. The emerging resistance of many gram positive and negative enteric pathogens continues to pose threat, yet such problem is unparallel with the discovery of alternative agents to battle the issue. Recently, much attention has been focused to unravel the medicinal properties of natural products and in tandem; the need to search for effective new antimicrobials continues to escalate[2]. Such interest may pave way to the discovery and invention of novel therapeutic agents that could benefit mankind[5].

Culinary herbs and their essential oils have been used extensively for many years in food products, perfumery, and dental and oral products due to their different medicinal properties[3]. However, secondary plant metabolites are largely unexploited in 'conventional' animal production systems. In the past, plant metabolites were generally considered as a source of anti-nutritional factors. Recent bans and restrictions on the use of animal antibiotic growth promoters stimulated interest in bioactive secondary metabolites of plant source as alternative performance enhancers[4]. '**Vigna aconitifolia**', commonly called moth bean or dew bean, sprouts are sprouts used in many medicinal preparations.

MATERIALS AND METHOD

Plant material:

The seed material of Vigna aconitifolia[6], was collected from surroundings of Aditya College of pharmacy, Kakinada, East Godavari. Sprouts were air dried ground to fine powder and stored in an airtight container at room temperature until further use. Exposure to light was kept to a minimum to prevent loss of active compounds.

Preparation of extract:

The powdered sample was subjected to extraction using organic solvent ethanol. 85g of powdered sprouts were macerated in 500 mL, of respective solvent for five days; the solvent mixture is filtered with the help of Watt man No.1 filter paper. The obtained filtrate is subjected to distillation[7]. The extract thus obtained is then dried used in present study.

Test organisms used:

The various organisms like Staphylococcus aureus (ATCCBAA 1026), Bacillus subtilis (ATCC 11774), Staphylococcus Werneri (ATCC 27836), Pseudomonas putida (ATCC 700007),

Pseudomonas aeruginosa (ATCC 10662), are procured from Microbes Speciality Lab Danavaipeta , Rajahmundry , East Godavari District 533103 , Andhra Pradesh , India.

Standardization of Micro-Organisms:

One loop full of micro-organisms were inoculated into 1000ml of sterile medium and incubated for 24hrs at 37ºc for bacterial culture and for 48hrs at 27ºc for fungal culture. After 24h/ 48hr of incubation[8], 1ml of broth containing the microorganisms was added to 9ml of peptone water.10 fold serial dilutions were made in the range of 10^{-1} to 10^{-10}.100µl of the dilutions ranging from 10^{-5} to 10^{-8} were spread over the sterile nutrient agar (SDA) plates and kept at 37 and 27ºc for 24/48 hours respectively[9,16]. The number of colony forming units (CFU) was counted and number of micro-organisms per 1ml of stock culture was calculated.

Antibacterial Agent:

The reference standard Gentamycin was procured from Pradeep Organics and chemicals Pvt. Ltd, Hyderabad. MIC for the most sensitive organism was determined by microdiluton method, in accordance to the national committee for clinical laboratory standards (NCCLS). MIC is defined as the lowest concentration of antimicrobial agent required to inhibit growth of the bacteria[10].

Preparation of test and standard solutions:

The stock solution of test compounds was prepare by dissolving the dried seed extract at a concentration of 100 and 300 and 500mg/ml. the stock solution of reference standard Gentamycin was prepared at a concentration of 25µg/ml in sterile water.

Antibacterial Assay:

Seed extract of Vigna aconitifolia was evaluated for antibacterial activity against several Gram Positive and Gram Negative organisms[15]. The antibacterial activity of ethanolic extract was performed using Agar cup-plate method[11]. 20ml of sterile nutrient agar medium was poured into sterile Petri-dishes and allowed to solidify. The Petri dishes were incubated at 37°C for 24 hours to check for sterility. The medium was seeded with the organisms by pour plate method using sterile top agar (4 ml) contained 1 ml culture[12]. Bores were made on the medium using sterile borer. Dried ethanolic extract of seeds of Vigna aconitifolia was dissolved in water to obtain different concentrations (100, 300 and 500mg/ml) and sterilized by filtration through a Whattman filter paper no. 1, and 0.05 ml of the different concentrations of extracts were added to the respective bores. 0.05ml of Gentamycin at a concentration of (25 µg/ml) was taken as standard reference[14]. All the plates were kept in a refrigerator at 2 to 8 o c for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37ºc for 24 hours. The presence of

definite zone of inhibition of any size around the cup indicated Antibacterial activity[13]. The diameter of the zone of inhibition was measured and recorded.

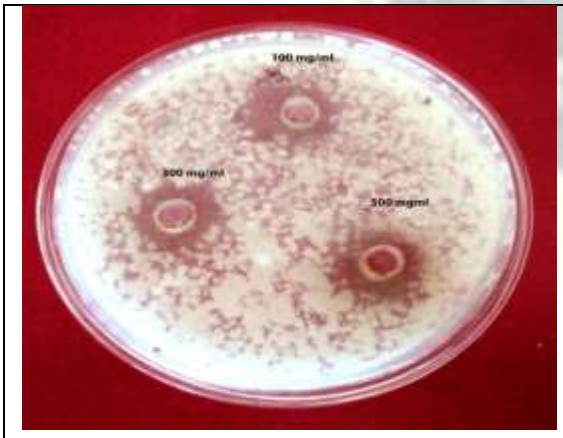
RESULTS AND DISCUSSION

The seed extract of Vigna aconitifolia was studied for antibacterial activity employing standard cylinder method. Microbes used were *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus werneri*, *Pseudomonas aeruginosa and Pseudomonas putida*. Both gram-positive and gram-negative bacteria were sensitive to the extract. The zone of inhibition recorded for various organisms was found *Staphylococcus aureus* (18mm), *Bacillus subtilis* (13mm), *Staphylococcus werneri* (14mm), *Pseudomonas putida* (17mm), *Pseudomonas aeruginosa* (15mm). Activity of ethanolic extract of the plant was comparable to that of Reference Standard drug Gentamycin disc (25µg). Vigna aconitifolia *seed* extract exhibited good antibacterial activity and results were tabulated along with figures.

Table 1: Antibacterial activity of *Vigna Aconitifolia* Extract

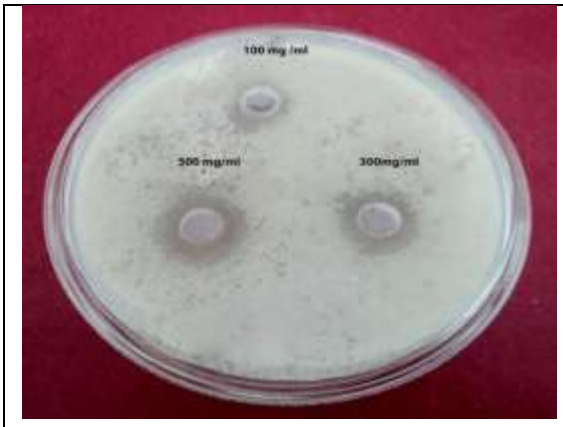
Zone of inhibition(mm)					
Microorganism	**100mg/ml**	**300mg/ml**	**500mg/ml**	**Gentamycin**	**25µg/ml**
Gram Positive					
S.aureus	16 ± 0.21	17 ± 0.36	18 ± 0.15	12 ± 0.12	
B.subtilis	10 ± 0.62	12 ± 0.69	13 ± 0.36	14 ± 0.32	
S.werneri	12 ± 0.48	14 ± 0.48	14 ± 052	13 ± 0.28	
Gram Negative					
P.putida	13 ± 0.26	13 ± 0.52	17 ± 0.25	15 ± 0.21	
P.aerugenosa	12 ± 0.36	13 ± 0.39	15 ± 0.53	16 ± 0.12	

Results are mean ±S.D. values of three. No activity (diameter of the inhibition zone less than 10mm)

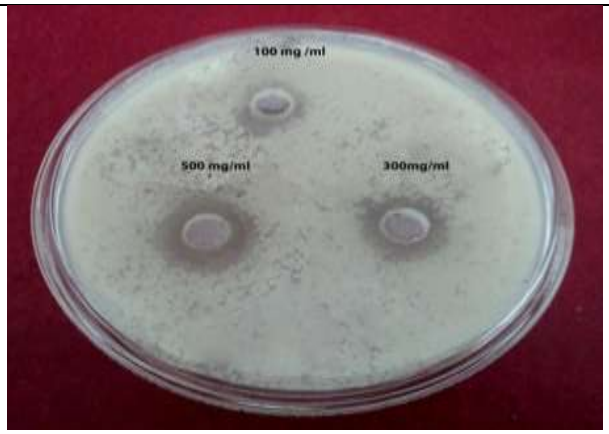


Staphylococcus aureus

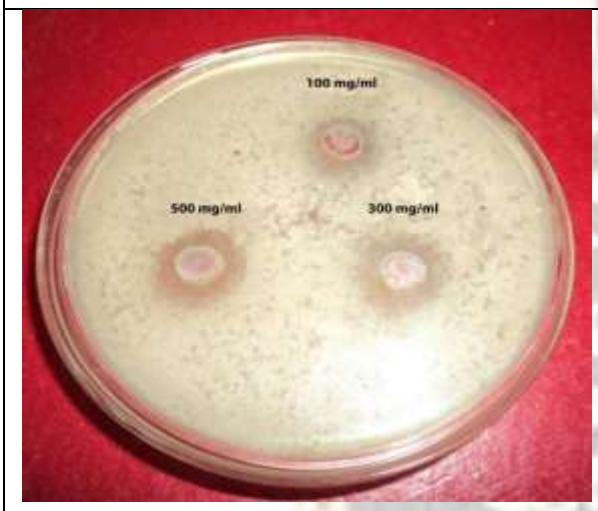
Bacillus subtilis



Staphylococcus werneri



Pseudomonas aeruginosa



Pseudomonas putida

Standard

Figure 1: *in vitro* antibacterial activity

The *in vitro* antibacterial activity showed that *Vigna aconitifolia* root extract possessed good significant antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Staphylococcus werneri, Pseudomonas putida, Pseudomonas aeruginosa, Proteus mirabilis* .The antibacterial activity of root extract with different concentrations 100 , 300 and 500mg/ml was very well compared with standard reference drug Gentamycin 25 µg/ml .The maximum zone of inhibition with extract of *Vigna aconitifolia* at various concentrations like 100 , 300 and 500mg/ml are 16,17 and 18 mm respectively.

CONCLUSION

Scientists have realized an immense potential in natural products from medicinal plants to serve as alternate source of combating infections in human beings which may also be of lower cost and lesser toxicity. Further investigations are required in order to isolate more new compounds from the plant extracts and to test their bioactivities with the aim of increasing the drug arsenal currently

used in the treatment and prophylaxis of human and animal diseases. It is very necessary to introduce new and biologically safe and active drugs eco-friendly in nature and effective as antibacterial agents. Usually medicinal plants contain several phytochemical compounds, which are very much necessary to control the growth of the micro organisms. In fine, this paper establishes that ***Vigna aconitifolia seed extract*** have good activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus werneri*, *Pseudomonas putida*, *Pseudomonas aeruginosa*. This plant can be further subjected to isolation of the therapeutic antibacterial and carryout further pharmacological evaluation. However, before coming to conclusive statement, further research is needed to investigate the antibacterial ingredients.

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