

## Original Research Article

# Effect of *Phyllodium Longipes* Leaf Extract on Multi Drug Resistant Bacteria

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**Abstract:** Long before the discovery of microbes, plants were well known for their healing potential against different diseases including infectious diseases. MDR (Multi Drug Resistant) bacteria are a source of major concern for clinicians as most primary antibiotics do not work against them. Due to this, there is a drastic increase in the number of infections caused by MDR strains which in turn leads to an increasing number of deaths due to lack of treatment. The aim of this study is to determine the anti-microbial properties of the extract of *Phyllodium longipes*, a lesser-known edible plant valued for its medicinal properties. However, its antimicrobial properties are largely unknown. In this study we explored its antimicrobial property against some pathogenic bacteria including MDR strains. The Minimum Inhibitory Concentration (MIC) of the ethanol extract of the plant was determined. This study shows that the plant extract is highly effective against Gram positive bacteria MRSA (Methicillin Resistant *Staphylococcus aureus*), Gram negative bacteria *Klebsiella pneumoniae* and *Salmonella typhi*, while it is mild to moderately effective against other pathogenic bacteria.

**Keywords:** *Phyllodium longipes*, MDR bacteria, *Staphylococcus aureus*, MRSA.

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## INTRODUCTION

*Phyllodium longipes*, is a perennial woody and leguminous shrub native to Southern China, Cambodia, Laos, Myanmar, Vietnam and Thailand, and found in India, and South eastern Asia, typically located in monsoon forests, grasslands and scrublands within sub-tropical and monsoonal climates. Belonging to the angiosperm division and classified as a dicotyledon this shrubby plant can grow up to 1-2 metres. *Phyllodium* is a genus of flowering plants in the family Fabaceae, in the subfamily Faboideae, tribe Desmodieae and subtribe Desmodinae. The genus name *Phyllodium* refers to flattened leaf like bracts enclosing the plant's flowers while the species name *longipes* means "long stalked" referring to the trailing inflorescence shoots. It features dense velvety brown hair on both its stems and foliage

with terminal leaves that are lanceolate to oblong and prominently veined. The plant is evergreen bearing smooth velvety foliage and bisexual flowers that are cream to white in color. It reproduces via dehiscent dry fruits (capsules) through explosive abiotic seed dispersal. *Phyllodium longipes* is autotrophic and thrives under full sun with moderate watering and a little bit of shade and is hardly able to withstand torrential monsoon rains, though it should not be placed in deep shade. It is commonly used in landscaping due to its ornamental foliage and is suitable for container planting, naturalistic gardens and butterfly gardens as it serves as a butterfly host plant. Its growth rate is moderate, and it possesses a woody stem with an underground root system comprising tap and fibrous roots. It thrives in fertile loamy and well-drained soil with moderate maintenance requirements.

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**Fig.1-Phyllodium longipes**

## MATERIALS AND METHODS

The plant was collected from Ayodhya Hills, Purulia, West Bengal and was identified by a botanist. The leaves were cleaned of extraneous material and the necrotic parts were removed and washed with clean water. The plant was then transferred to the laboratory in polythene bags. The plant was thoroughly washed with running water followed by a wash with distilled water to get rid of any dirt.

Microbial Cultures – *E.coli* ATCC 25922, *E.coli* MDR, *Klebsiella pneumoniae* MDR, *Pseudomonas aeruginosa* MDR, *Staphylococcus aureus*

ATCC 25923, *Staphylococcus aureus* MRSA and *Salmonella typhi* were isolated from infected human sources and suspended in saline solutions and centrifuged.

Preparation of the plant extract – the leaves were separated from the stem and diced into small pieces using a clean blade. These pieces were then weighed on a weighing balance. 2 grams of the cut pieces were measured and were added to 10 ml of 70% alcohol. This mixture was swirled in vortex to obtain the extract and was then left undisturbed in a dark place for 72 hours. All these procedures were performed in aseptic conditions.



**Fig.2-P.longipes extract**

Mueller Hinton Broth – 4.2 gram of Mueller Hinton Broth powder was added to 200ml of distilled and water, mixed well and then autoclaved. Post autoclaving, the sterile media was stocked in the laboratory for performing the experiment.

## METHODOLOGY

The bacterial strains were first subcultured on respective solid media to obtain the pure strains. Then the inoculum from this culture was added to a saline suspension and centrifuged for creating a homogenous

mixture. After this, the microtiter plate was taken and 100 microliters of Mueller Hinton Broth were pipetted into each well of this plate. Then 100 microliters of plant extract were pipetted to the 1<sup>st</sup> well of the microtiter plate and mixed well. 100 microliters of this mixture were then taken and added to the second well located horizontally to the 1<sup>st</sup> well and mixed. Then the same procedure was repeated for the 1<sup>st</sup> row up until the 8<sup>th</sup> well. 100 microliters of the mixture from the 8<sup>th</sup> well were discarded after proper mixing to equalize the amounts of mixture present on the 1st row. The 2nd row of the microtiter plate was for making control. 100 microliters of alcohol were added to the 1st well of the 2nd row and then serially diluted as mentioned before up until the 8th well. This row was done as a control to compare the results. Hence, rows 1,3,5,7,9 and 11 had plant extracts while rows 2,4,6,8,10 and 12 were for their respective controls. Then, 10 microliters of *E.coli* ATCC was pipetted out from the saline suspension and added to the all the wells of the 1<sup>st</sup> and 2 row and mixed properly. 10 microlitre of *E.coli* MDR was added to all the wells of 3<sup>rd</sup> and 4<sup>th</sup> rows. 10 microliters of *Klebsiella pneumoniae* MDR suspension was added to all the wells of the 5<sup>th</sup> and 6<sup>th</sup> rows. 10 microliters of *Psuedomonas aeruginosa* MDR was added to all the wells of the 7<sup>th</sup> and 8<sup>th</sup> rows.

10 microliters of *Staphylococcus aureus* suspension were added to all the wells of the 9<sup>th</sup> and 10<sup>th</sup> rows. Finally, 10 microliters of *Staphylococcus aureus* MRSA suspension was added to all the wells of 11<sup>th</sup> and 12<sup>th</sup> rows.

After completion of this process the plate was rotated to mix the contents properly. An initial reading was taken at 0 hour at 620 nm using a MicroScan.

After 24 hours another reading was taken, and the baseline reading was deducted from the final reading of each well.

## RESULTS

This study shows that the plant extract is highly effective against Gram positive bacteria MRSA (Methicillin Resistant *Staphylococcus aureus*), Gram negative bacteria *Klebsiella pneumoniae* and *Salmonella typhi*, while it is mild to moderately effective against other pathogenic bacteria.

### For *Staphylococcus aureus* ATCC 25923

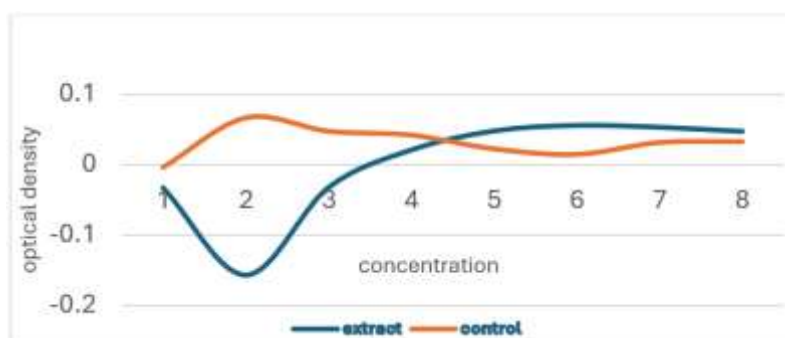


Fig. 3: Showing MIC value of extract against *Staphylococcus aureus* ATCC 25923. Concentration of extract: 1= 25mg/ml; 2= 12.5mg/ml; 3=6.25mg/ml; 4= 3.125mg/ml; 5= 1.56mg/ml; 6= 0.78mg/ml; 7= 0.39mg/ml. MIC value 3.125mg/ml

### Methicillin For Resistant *Staphylococcus aureus* (MRSA)

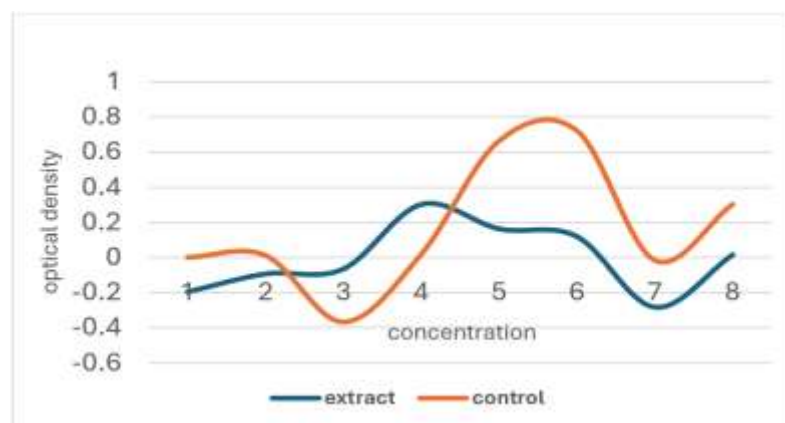
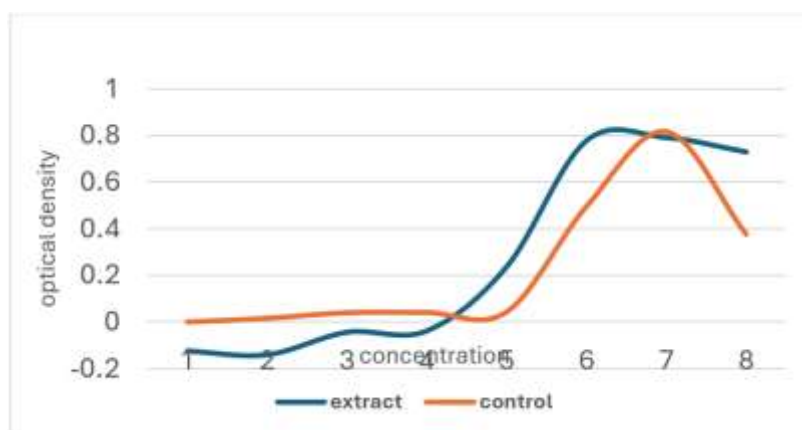


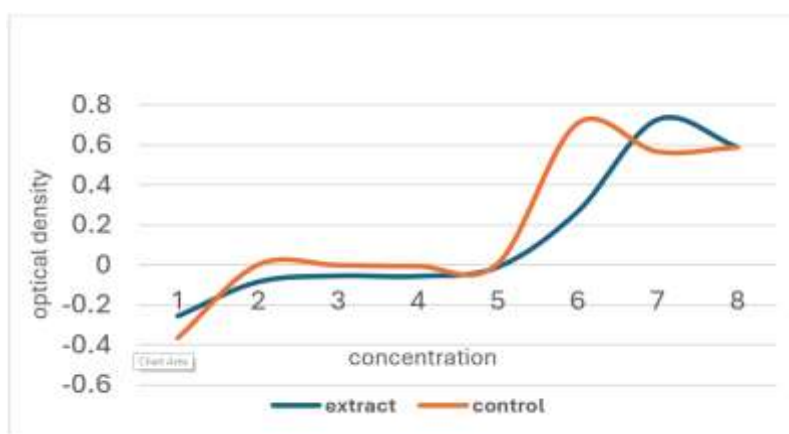
Fig. 4: Showing MIC value of extract against MRSA Methicillin Resistant *Staphylococcus aureus* Concentration of extract: 1= 25mg/ml; 2= 12.5mg/ml; 3=6.25mg/ml; 4= 3.125mg/ml; 5= 1.56mg/ml; 6= 0.78mg/ml; 7= 0.39mg/ml; 8= 0.19mg/ml. MIC Value <0.19mg/ml.

**For *E.coli* MDR (Multi Drug Resistant)**



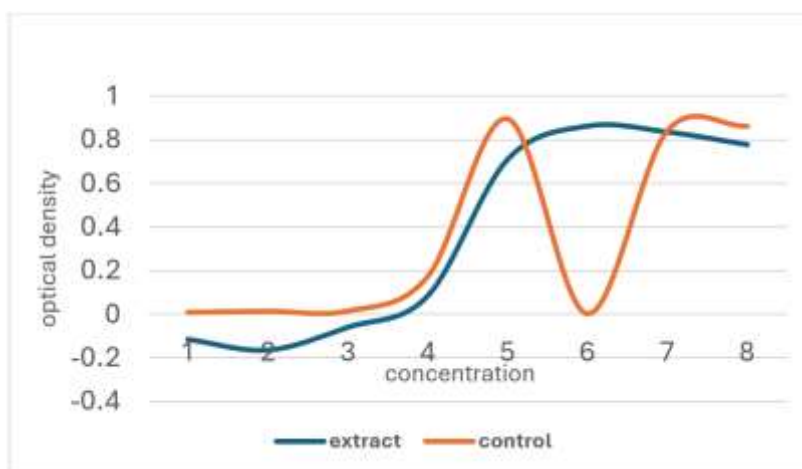
**Fig. 5: Showing MIC value of *Escherichia coli* MDR Concentration of extract: 1= 25mg/ml; 2= 12.5mg/ml; 3=6.25mg/ml; 4= 3.125mg/ml; 5= 1.56mg/ml;6= 0.78mg/ml; 7= 0.39mg/ml. MIC Value: 3.125mg/ml.**

**For *E.coli* ATCC 25922**



**Fig. 6: Showing MIC Value of *Escherichia coli* ATCC 25922; Concentration of extract: 1= 25mg/ml; 2= 12.5mg/ml; 3=6.25mg/ml; 4= 3.125mg/ml; 5= 1.56mg/ml; 6= 0.78mg/ml; 7= 0.39mg/ml; 8=0.19mg/ml. MIC Value: 0.78mg/ml**

**For *Klebsiella pneumoniae* MDR (Muti Drug Resistant)**



**Fig. 7: Showing MIC Value of *Klebsiella pneumoniae* MDR. Concentration of extract: 1= 25mg/ml; 2= 12.5mg/ml; 3=6.25mg/ml; 4= 3.125mg/ml; 5= 1.56mg/ml; 6= 0.78mg/ml; 7= 0.39mg/ml; 8= 0.19mg/ml. MIC Value: <0.19mg/ml.**

### For *Pseudomonas aeruginosa* MDR (Multi Drug Resistant)

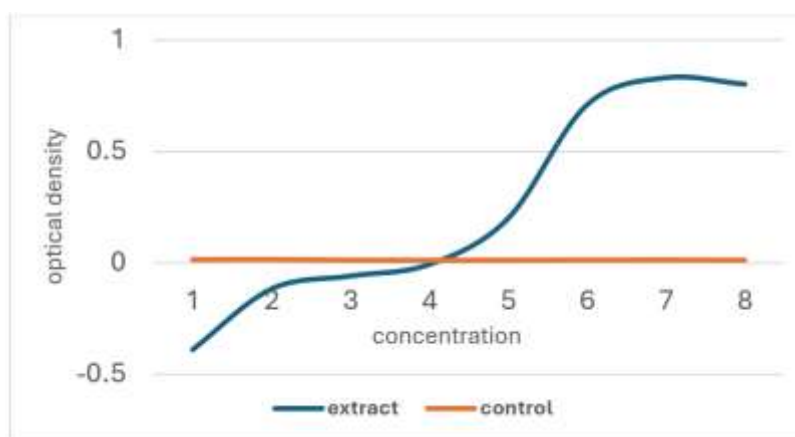


Fig. 8: Showing MIC Value of *Pseudomonas aeruginosa* MDR. Concentration of extract: 1= 25mg/ml; 2= 12.5mg/ml; 3=6.25mg/ml; 4= 3.125mg/ml; 5= 1.56mg/ml; 6= 0.78mg/ml; 7= 0.39mg/ml; 8= 0.19mg/ml. MIC Value: <3.125mg/ml.

### For *Salmonella typhi*

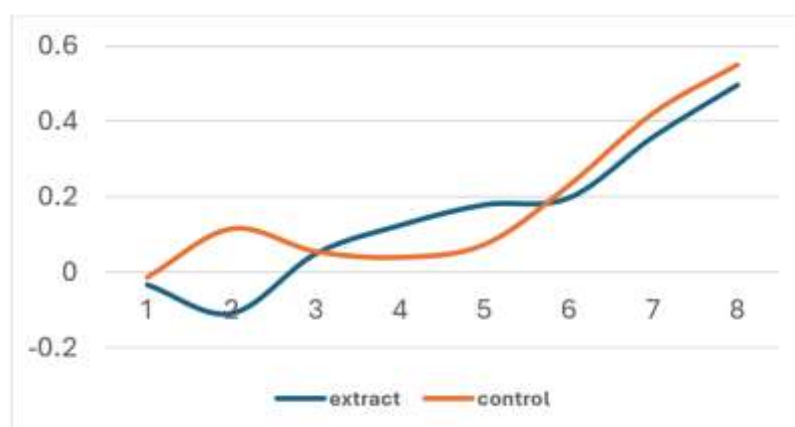


Fig. 9: Showing MIC Value of *Salmonella typhi*. Concentration of extract: 1= 25mg/ml; 2= 12.5mg/ml; 3=6.25mg/ml; 4= 3.125mg/ml; 5= 1.56mg/ml; 6= 0.78mg/ml; 7= 0.39mg/ml; 8= 0.19mg/ml. MIC value <0.19mg/ml

## DISCUSSION

Plants have been used from time immemorial for their medicinal properties, for flavoring and conserving food. The knowledge about their utility in treating diseases has been passed down through centuries among human communities (Silva and Fernandes, 2010). Most diseases are linked to the production of free radicals which are an integral part of aerobic life and metabolism which can cause extensive damage to human health leading to the development of a potential disease. Antioxidants help in treating damage caused by free radicals. Plants are known to possess antioxidants which can help in treating the damage conferred by antioxidants (Onyenibe Sarah Nwozo, 2023).

Phyllodium is a small genus of the tribe Desmodieae found distributed in India, Eastern and South-Eastern Asia and with a few species in Northern Australia. Species of this plant are known to exhibit a range of bioactivities such as anti-microbial, antioxidant, anti-inflammatory and various other properties (Vinayaka Ks, 2021).

## CONCLUSION

The present study evaluated the antibacterial efficacy of the plant extract against a wide range of both standard and multidrug-resistant (MDR) bacterial strains which include *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and MDR, MRSA, *Klebsiella pneumoniae* (MDR) and *Pseudomonas aeruginosa* (MDR) and *Salmonella typhi*. The results demonstrated a notable variation in susceptibility against the aforementioned organisms.

Among all, MRSA exhibited the highest sensitivity to the extract along with *Klebsiella pneumoniae* and *Salmonella Typhi*. ATCC *Staphylococcus aureus* and other tested bacteria in this experiment showed a notable response as well, albeit with a slightly higher MIC suggesting that the extract retained partial effectiveness against standard ATCC strains as well as some MDR strains resistant pattern. *Salmonella typhi* exhibits moderate sensitivity to the extract with a gradual decline in optical density observed

at higher concentrations indicating partial inhibition of bacterial growth.

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