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Antibacterial activity of different extracts of various parts of *Mucuna pruriens*

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Abstract

The current study aims to investigate the antibacterial efficacy of different parts of *Mucuna pruriens*, commonly referred to as "velvet bean", against various bacterial strains, such as *E. coli*, *S. aureus*, *Bacillus*, and *Pseudomonas aeruginosa*. The study showed obtaining of extracts from the roots, stems, leaves, and fruits, by usage of various solvents such as distilled water, methanol, chloroform, and petroleum ether. This was followed by determination of antibacterial efficacy of different extracts against as *E. coli*, *S. aureus*, *Bacillus,* and *Pseudomonas aeruginosa*. The results of the study revealed that the root extract of the plant exhibited potent antibacterial activity, which was observed particularly against *E. coli* and *Bacillus*, with the highest antibacterial efficacy being observed when chloroform was used for extraction. Conversely, the fruit and stem extracts demonstrated antibacterial efficacy against.

S. aureus. However, none of the extracts displayed substantial antibacterial activity against *Pseudomonas*. The findings of the study highlight the varying antibacterial efficacy of *Mucuna pruriens* based on plant part as well as solvent choice, suggesting its potential as a source of natural antibacterial agents. However, further research is needed to identify the specific bioactive compounds needed for these effects as well as to explore their mechanisms of action, contributing to the discovery of alternative therapies in the crusade against antibiotic-resistant bacteria.

Keywords: Antibacterial, Bacillus subtilis, E. coli, Mucuna pruriens, Pseudomonas aeruginosa, S. aureus, etc.

Introduction

The rising cases of antimicrobial resistance have become a pressing global health issue due to their serious health implications and inability to be treated effectively. The last few decades have witnessed burgeoning numbers of bacteria such *E. coli*, *S. aureus*, and *Pseudomonas aeruginosa* become increasingly resistant to conventional antibiotics. This has necessitated the requirement for searching new sources of antibacterial agents in order to restrain the increasing numbers. This has led to increased interest is the exploration of plant-based antibacterial compounds, which have been used in traditional medicine for centuries and offer a potential alternative to synthetic drugs [1-3].

The current study focussed on one such plant, named as *Mucuna pruriens*, commonly known as velvet bean. This leguminous plant survives in tropical as well as subtropical regions and is celebrated not only for its nutritional benefits but also for its medicinal properties. Traditionally, the plant *Mucuna pruriens* is used to treat a variety of health issues, ranging from digestive disorders to cardiovascular conditions and neurological conditions such as Parkinson's disease. Recent research over the last few decades has begun to uncover its antibacterial potential, revealing the presence of various bioactive compounds—such as alkaloids, flavonoids, as well as tannins—that may help in combating bacterial infections [4-6].

Different parts of the plant *Mucuna pruriens* such as the roots, leaves, stems, as well as fruits— may possess different antibacterial effects, which may occur due to variation in phytochemical profile as well as the unique composition of phytochemicals in each part of the plant. For instance, the presence of specific alkaloids in plant roots enable them to be particularly effective against certain bacteria, whereas the leaves of the plant might contain other compounds that offer other benefits. In addition to this, the phytochemical profile of each plant part is also affected by the choice of the solvent used for the extraction process. It is due to this that usage of solvents such as methanol, chloroform, as well as petroleum ether plays a significant role in affecting solubility as well as availability of these bioactive ingredients, ultimately impacting their antibacterial efficacy [7-10].

Considering this, the current study aims to explore the antibacterial efficacy of different parts of *Mucuna pruriens* against different bacterial strains, including *E. coli*, *S. aureus*, *Bacillus*, and *Pseudomonas*. By investigating the efficacy of plant extracts from the roots, stems, leaves, and fruits using different solvents, we aim to highlight the potential of *Mucuna pruriens* to serve as natural source of antibacterial agents. It is important to understand that which part of the plant work best against specific bacteria and how the employed extraction methods influence their antibacterial efficacy. Understanding these two basic phenomena would contribute towards harnessing the full therapeutic potential of this plant. The significance of the current study goes beyond mere academic curiosity and aligns with global efforts towards addressing the monstrous problem of antibiotic resistance. Owing to growing concerns about the side effects of synthetic antibiotics as well as their inherent limitations, there is an increasing demand for natural alternatives in order to effectively treat infections without promoting resistance. Therefore, the findings of the current research would not only validate the effectiveness of traditional plants through scientific research, but also bridge the gap between folklore as well as contemporary pharmacology. Therefore, the current study not only aims to highlight the antibacterial potential of *Mucuna pruriens* but also encourages its usage in development of novel strategies for tackling antibiotic resistant bacterial strains.

Materials and methods
Collection of plant and processing
Different parts (stem, leaves and fruits) were collected from Jaipur, Rajasthan. Those were identified by taxonomist and specimen was deposited at Herbarium, Department of Botany, university of Rajasthan, Jaipur.

The plant parts were washed first with running tap water to remove impurities and then with distilled water and shade dried. After that, those were grinned to make coarse powder and stored for further use.

Extraction of plant parts
One gram of each dried plant material was taken and dipped into 10 ml of different solvent.

Solvents were distilled water (polar), methanol, chloroform (mid polar) and petroleum ether (non polar). Extraction was done in sonicator. After filtration, solvents were evaporated and dry extracts were dissolved in DMSO to make concentration of 100 µg/ml.

Evaluation of Antibacterial activity
The antibacterial activity of plant extracts was performed by well diffusion method**.** *E. coli*, *S. aureus, Pseudomonas aeruginosa* and *B. subtilis* bacterial cultures were sub-cultured in Nutrient Agar and incubated at 37°C for 24 hours. Then the cultures were swabbed onto petriplates containing nutrient agar using sterilized cotton swab. Wells of 6 mm in diameter were punctured onto the agar plates and 30 µl of plant extracts were loaded into wells. The plates were incubated and the zone of inhibition of each well was measured. Streptomycin (100 µg/ml) was used as control to compare the effectiveness of plant extracts against tested bacteria.

Activity index was calculated by diving inhibition zone of test sample by inhibition zone by antibiotic drug. The experiments were conducted in triplicates.

Results and discussion
Antibacterial activity of different parts of *Mucuna pruriens* against *E. coli*
Escherichia coli (*E. coli*) is naturally residing in the intestines of humans and animals, playing a role in digestion. While several strains are harmless and even beneficial, some can cause serious illnesses. Several pathogenic strains of *E. coli* have been reported including Enterotoxigenic *E. coli* (causative agent of traveler's diarrhea.), Enteropathogenic *E. coli*, Enterohemorrhagic *E. coli, Enteroinvasive E. coli* and Uropathogenic *E. coli* [11].

The results in Table 1 and Figure 1 showcase antibacterial activity of 100 µg/l of root, stem, leaf and fruit extract of *Mucuna pruriens* obtained using different solvents (Distilled water, methanol, chloroform and Pet. Ether) against *E. coli*. The results indicate potent antibacterial efficacy of all the tested extracts against *E.coli*, with maximum antibacterial efficacy in case of root extract followed by leaf, stem and fruit extract. The highest antibacterial efficacy in case of both root and leaf extract was obtained when chloroform was used as a solvent. However, stem and fruit extract displayed maximum antibacterial efficacy when petroleum ether was used as a solvent. The order of diameter of inhibition zone (indicative of antibacterial efficacy of the plant extracts) was as follows:

Root extract>Leaf extract> Stem extract>Fruits extract

Root: Chloroform extract>Methanol extract>Distilled water extract>Petroleum ether extract

Stem: Distilled water extract=Petroleum ether extract>Methanol extract=chloroform extract

Leaves: Chloroform extract> Methanol extract>Distilled water extract>Petroleum ether extract

Fruits: Petroleum ether extract>Chloroform extract=Methanol extract> Distilled water extract

All the tested extracts demonstrated significant antibacterial efficacy against *E. coli*., with highest antibacterial efficacy in case of root extract. This indicates the presence of highest concentration of antibacterial bioactive secondary metabolites in roots of the plant. Furthermore, the highest antibacterial effect was observed when chloroform and methanol were used for extraction process, indicating the efficacy of these solvents to extract potent antibacterial phytochemicals from different plant parts, including roots and leaves. Stem and fruits showed lowest antibacterial efficacy, which shows that both stem and fruits contain low concentration of antibacterial phytochemicals and inability of solvents to extract these phytochemicals. The antibacterial efficacy of different parts of *Mucuna pruriens* maybe attributed to presence of several secondary metabolites such as alkaloids, flavonoids, triterpenes, saponins, phenolic compounds as well as several essential oils, all of which have been reported to showcase potent antibacterial efficacy. Several other studies have also demonstrated potent antibacterial efficacy of different plant parts of *Mucuna pruriens* [12-15].

Antibacterial activity of different parts of *Mucuna pruriens* against *S. aureus*
Staphylococcus aureus, a Gram-positive bacterium, commonly lives on our skin and noses. Most of the times, this bacterium is harmless, however, some of the bacterial strains can be quite dangerous and lead to a range of infections, including minor skin issues to severe health complications, ranging from skin infections such as boils, cellulitis, abscesses, respiratory infections, bone and joint infections, endocarditis and food poisoning. *Staphylococcus aureus* is one of the biggest menace in current scenario owing to antibiotic resistance, leading to emergence of Methicillin-resistant *Staphylococcus aureus* (MRSA) strain [16].

The results in Table 2 and Figure 2 showcase antibacterial activity of 100 µg/l of root, stem, leaf and fruit extract of *Mucuna pruriens* obtained using different solvents (Distilled water, methanol, chloroform and Pet. Ether) against *S. aureus* The results indicate maximum antibacterial efficacy in case of fruit extract when petroleum ether was used as a solvent. However, fruit extract displayed no potent antibacterial effect in cases when water, methanol or chloroform was used as solvent. The second highest antibacterial efficacy was displayed by stem extract (petroleum ether extract) and leaf extract (chloroform extract). The root extract did not show any antibacterial efficacy.

The order of diameter of inhibition zone (indicative of antibacterial efficacy of the plant extracts) was as follows:

Fruit extract> Stem extract>Leaf extract>Root extract

Stem: Petroleum ether extract> Methanol extract=Chloroform extract>Distilled water extract

Leaves: Chloroform extract>Methanol extract> Distilled water extract> Petroleum ether extract

Fruits: Petroleum ether extract>Methanol extract= Chloroform extract=Distilled water extract In complete contrast with what was observed in case of *E. coli*, the highest antibacterial efficacy against S. aureus was observed in case of fruit extract followed by nearly similar antibacterial efficacy in case of stem and leaf extracts. Contrastingly, root extract, which was found to be most effective in neutralizing gram-negative *E. coli,* showed no visible antibacterial efficacy against the gram-positive *S. aureus*. This may be attributed to variability in structure of cell wall of *E.coli* and *S. aureus*, wherein, *E. coli* being a Gram-negative bacterium possesses thin peptidoglycan layer and outer membrane, whereas *S. aureus* being Gram-positive bacterium possesses thicker peptidoglycan layer with no outer membrane. This structural difference account for variability in ways in which antibacterial compounds penetrate the cell wall, leading to variation in effectiveness of extracts against each type of bacteria. Therefore, bioactive compounds present in roots, stem, fruit and leaves may differ in their efficacy against peptidoglycan layer of *S. aureus*, while others may target the outer membrane of *E. coli*.

Several other studies have also demonstrated potent antibacterial efficacy of different plant parts of *Mucuna pruriens* against *S. aureus* [17-20].

Antibacterial activity of different parts of *Mucuna pruriens* against *Bacillus subtilis*

The genus *Bacillus* includes a whole diverse group of Gram-positive, rod-shaped bacteria that commonly occur in soil, water, as well as in our digestive tracts. Most of the species of *Bacillus* are harmless or even beneficial for that matter, however, a few can cause serious health illnesses. Pathogenic strains of Bacillus include *Bacillus anthracis* (causative agent of anthrax and causes *Cutaneous anthrax,* inhalational anthrax and gastrointestinal anthrax), *Bacillus cereus* and *Bacillus thuringiensis* [21].

The results in Table 3 and figure 3 showcase antibacterial activity of 100 µg/l of root, stem, leaf and fruit extract of *Mucuna pruriens* obtained using different solvents (Distilled water, methanol, chloroform and Pet. Ether) against *Bacillus subtilis*. The results show that none of the plants showed antibacterial efficacy against Bacillus, except root extract obtained via using chloroform and methanol as solvent. The highest antibacterial efficacy of root extract was observed when chloroform was used as solvent followed by methanol. This may occur due to the fact that different parts of *Mucuna pruriens* may contain varying concentrations as well as types of bioactive compounds. The roots of the plant may contain specific compounds that exhibit antibacterial activity against *Bacillus subtilis* but the other plant parts may be lacking those compounds or showing their presence in lower amounts in the stem, leaf, or fruit, which makes it difficult to extract these compounds using the mentioned solvents. Another reason for this maybe that the concentration of active compounds in the chloroform as well as methanol extracts from the root may have reached a threshold concentration that is sufficient to inhibit growth of Bacillus, whereas other parts did not provide similar concentrations or bioavailability of active compounds. However, A recent study [6] showed the antibacterial activity of *Mucuna pruriens* seed extract functionalized zinc oxide nanoparticles (ZnO NPs) against *Bacillus subtilis.*

Antibacterial activity of different parts of *Mucuna pruriens* against *Pseudomonas aeruginosa.*

The *Pseudomonas* genus includes a number of Gram-negative bacteria commonly found in soil, water, and even in our bodies. Most of the *Pseudomonas* species are harmless, however, *Pseudomonas aeruginosa* stands out as a major health concern owing to its ability to cause serious infections in immunocompromised individuals with chronic health issues. *Pseudomonas aeruginosa* has been reported to cause several respiratory infections, skin infections, urinary tract infections as well as bacteremia [22].

The results in Table 4 and Figure 4 showcase antibacterial activity of 100 µg/l of root, stem, leaf and fruit extract of *Mucuna pruriens* obtained using different solvents (Distilled water, methanol, chloroform and Pet. Ether) against *Pseudomonas***.** None of the plant extracts using different solvent mixtures displayed potent antibacterial activity against *Pseudomonas.*

The absence of antibacterial activity from *Mucuna pruriens* extracts against *Pseudomonas* may occur due to various reasons. Firstly, *Pseudomonas aeruginosa* is particularly resistant, showcasing presence of strong resistance mechanisms such as efflux pumps as well as tough outer membrane, all of which make antibacterial compounds difficult to penetrate. Additionally, the specific compounds in the plant may not be able to target the structures or processes of *Pseudomonas*, suggesting that the extracts lack the correct ingredients needed to fight this bacterium.

Also, the choice of solvents used for extraction such as distilled water, methanol, chloroform, and petroleum ether may also play a role. If the solvents don't effectively extract the necessary compounds, the resulting extracts will simply not have the potency required to combat *Pseudomonas*. Additionally, different parts of the plant may contain compounds that work well against other bacteria but are ineffective against *Pseudomonas*, highlighting a kind of specificity in how these extracts function. The concentrations of active compounds present in the extracts might also be too low to make a significant impact, meaning even if there are some beneficial ingredients, they aren't enough to inhibit bacterial growth. Lastly, the environmental conditions in which *Mucuna pruriens* grows could affect the production of its secondary metabolites, leading to variations in its antibacterial properties. All these factors combine to explain why *Mucuna pruriens* extracts showed no antibacterial activity against *Pseudomonas*.

In contrast to what we observed, a study [23] showed potent antibacterial efficacy of combination of leaf methanolic extracts of *Evolvulus alsinoides* and *Mucuna pruriens* against *Pseudomonas aeruginosa.* This antibacterial efficacy of the plant extracts was attributed to the presence of pharmacologically activity phytochemicals including alkaloids, flavonoids, and tannins. In yet another study, researchers[24] showed antibacterial efficacy of aqueous and ethanolic extract obtained from seed husk of *Mucuna pruriens* against *Pseudomonas sp.* BB22, which was attributed to the presence of Flavonoids, phenol, glycosides, steroids, saponins and anthraquinones.

Table 1: Antibacterial activity of different plant extracts against *Escherichia coli.*

Plant part	Water	Methanol	Chloroform	Pet ether	Standard
Root	**13**	**17**	**18**	**12**	**35**
Stem	**12**	**11**	**11**	**12**	**35**
Leaves	**13**	**12**	**14**	**10**	**35**
Fruits	**11**	**13**	**13**	**14**	**35**

Table 2: Antibacterial activity of different plant extracts against *S. aureus.*

Plant part	Water	Methanol	Chloroform	Pet ether	Standard
Root	NA	NA	NA	NA	39
Stem	NA	11	11	12	39
Leaves	10	11	12	NA	39
Fruits	NA	NA	NA	26	39

Table 3: Antibacterial activity of different plant extracts against *Bacillus subtilis.*

Plant part	Water	Methanol	Chloroform	Pet ether	Standard
Root	NA	8	15	NA	40
Stem	NA	NA	NA	NA	40
Leaves	NA	NA	NA	NA	40
Fruits	NA	NA	NA	NA	40

Table 4: Antibacterial activity of different plant extracts against *Pseudomonas aeruginosa.*

Plant part	Water	Methanol	Chloroform	Pet ether	Standard
Root	NA	NA	NA	NA	40
Stem	NA	NA	NA	NA	40
Leaves	NA	NA	NA	NA	40
Fruits	NA	NA	NA	NA	40

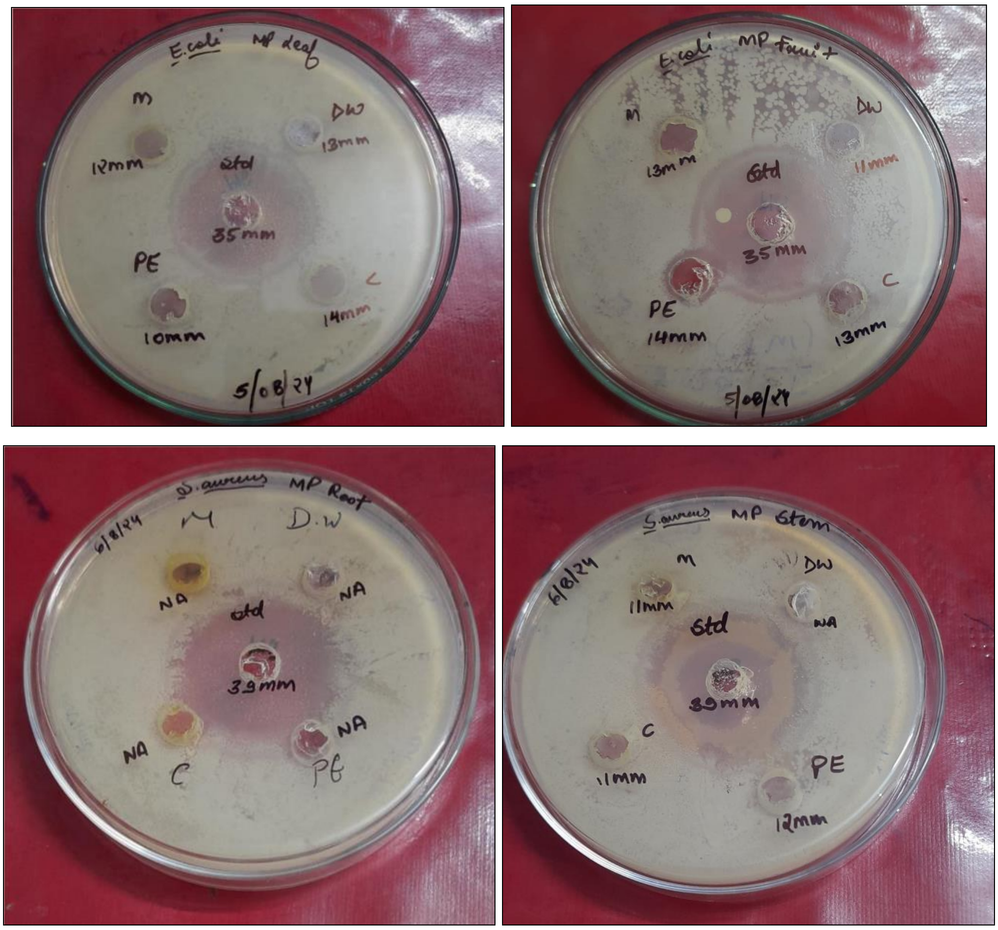


Fig 1: Antibacterial activity of different parts of *Mucuna pruriens* against *E. coli.*

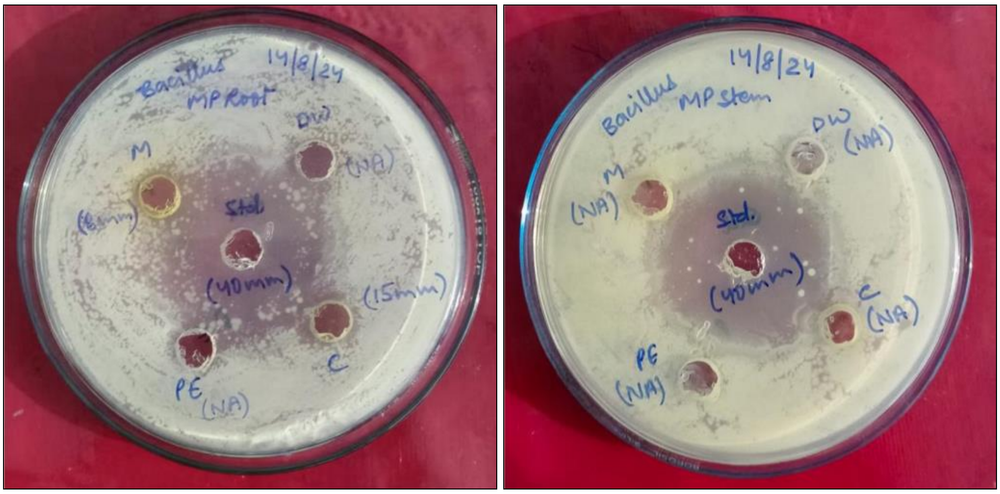


Fig 2: Antibacterial activity of different plant extracts against *S. aureus.*

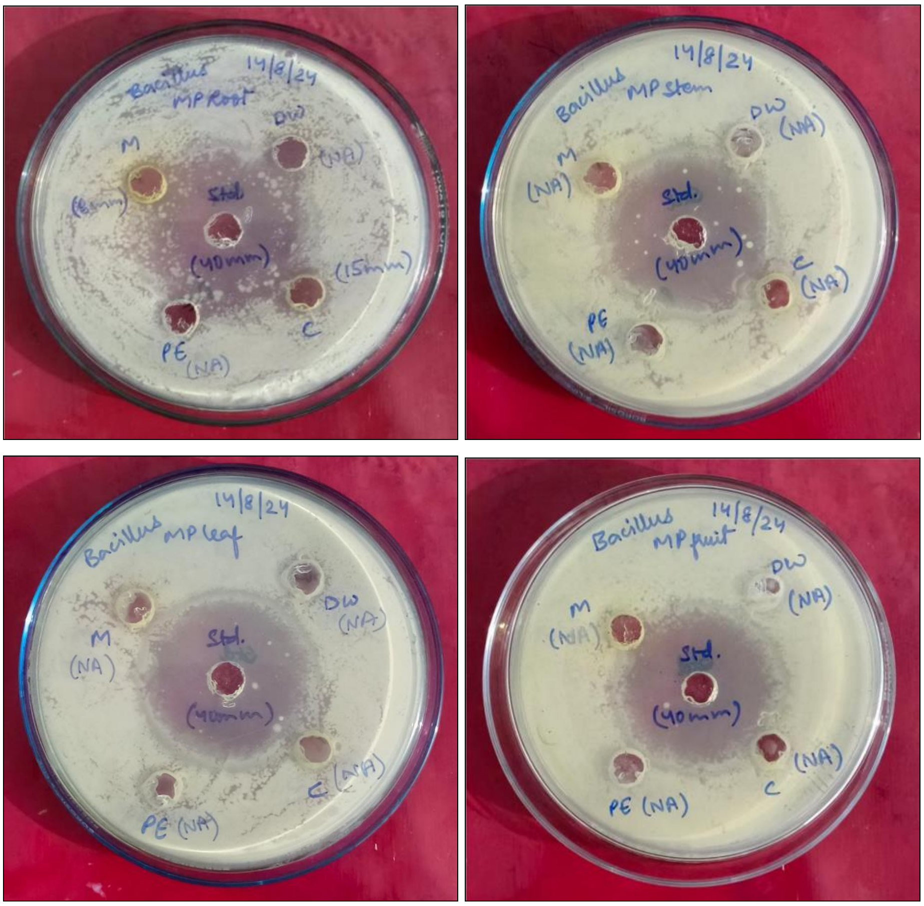
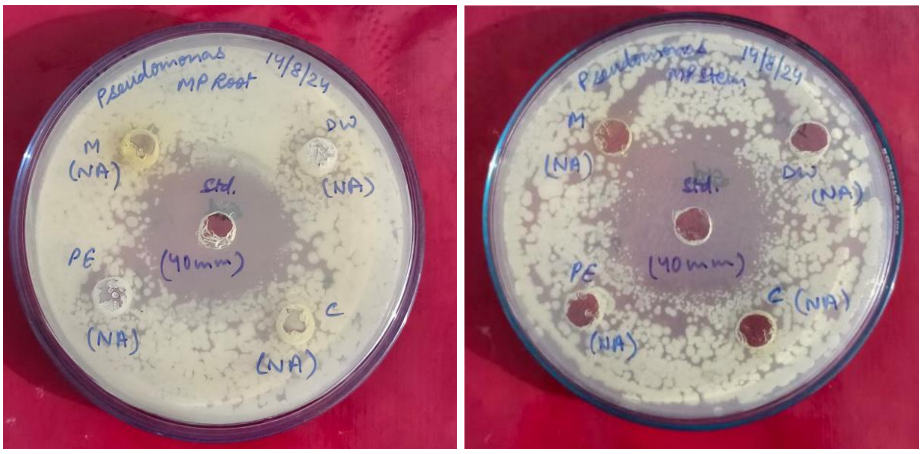


Fig 3: Antibacterial activity of different plant extracts against *Bacillus subtilis.*



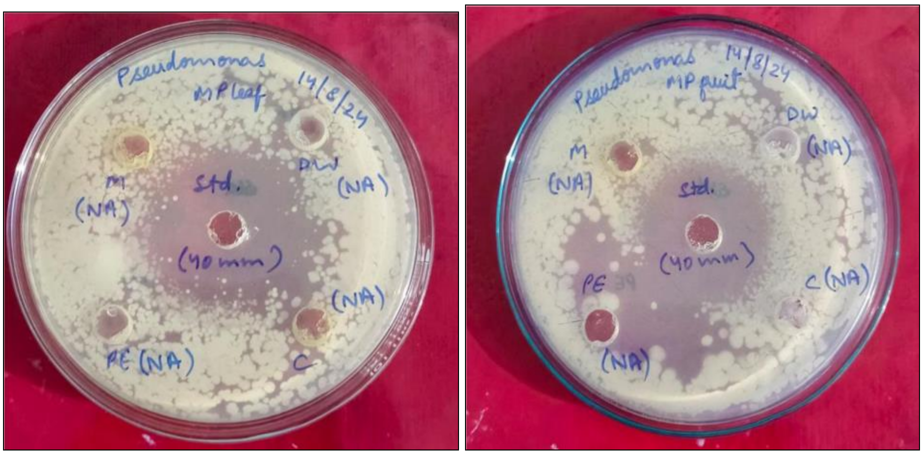


Fig 4: Antibacterial activity of different plant extracts against *Pseudomonas aeruginosa.*

Conclusion

In conclusion, this current study highlights the varying antibacterial efficacy of different parts of *Mucuna pruriens* against various bacterial pathogens, including *E. coli*, *S. aureus*, *Bacillus*, and *Pseudomonas aeruginosa*. The results of the study indicate that the root extract demonstrated the highest potent antibacterial efficacy, particularly against *E. coli* and *Bacillus*, especially when chloroform was used as solvent for extraction. In complete contrast to this, *S. aureus* showed sensitivity primarily to the fruit as well as stem extracts, whereas no significant activity was observed against *Pseudomonas aeruginosa* from any part of the plant. These findings highlight the importance of both the plant part as well as the choice of solvent in determining antibacterial efficacy of different plant extracts. The findings of the current study suggest that *Mucuna pruriens* showcases the potential to be used as source of natural antibacterial agents, particularly the root and fruit extracts of the plant. However, further research is needed to identify the specific bioactive compounds which are responsible for antibacterial effects of the plant and to explore their mechanisms of action. Overall, *Mucuna pruriens* appears to be a promising candidate for future development in the field of natural antibacterial therapies.

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