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ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF VARIOUS PARTS OF *PEDILIUM MUREX*.

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This current study investigates the antibacterial properties of extracts from different parts of the plant *Pedilium murex*, focusing on their efficacy against several bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus*, and *Pseudomonas aeruginosa*. With increase in number of antibiotic-resistant bacterial strains, the current study sought to investigate alternative sources of antimicrobial agents in the form of plant-derived compounds. The study begins with preparation of extracts from the leaves, stems, and flowers of *Pedilium murex* using different solvents—distilled water, methanol, chloroform, and petroleum ether—at a concentration of 100 µg/l. The results of the study showed that leaf extracts were particularly potent in controlling the bacterial infection by *Escherichia coli*, *Staphylococcus aureus*, *Bacillus*, especially when chloroform or petroleum ether was used as a solvent. However, in contrast to this, the stem and flower extracts showed minimal antibacterial activity against the tested bacterial strains. Notably, *Pseudomonas aeruginosa* was found to be resistant to all the extracts, highlighting the challenges in treatment of infections by this pathogen. The current study underscores the importance of solvent choice in extraction of bioactive compounds as well as their varying efficacy against specific bacteria. Overall, *Pedilium murex* has been shown to be a promising natural source of antibacterial agents, especially for combating infections caused by *E. coli*, *S. aureus*, and *Bacillus*. The findings of the current study will contribute to deeper understanding of plant based antimicrobial agents while also highlighting their potential role in overcoming the growing problem of antibiotic resistance.

Keywords: Antibacterial activity, *Pedilium murex*, Plant parts, Solvent extraction, Well diffusion assay.

Introduction

The last few decades have witnessed emergence of problem of antibiotic resistance at a global level, which has become major concern for public health today. Looking at the current situation and witnessing dwindling of conventional antibiotics lose their efficacy against various disease-causing bacteria has taken the entire scientific and healthcare community by a storm. The current anarchial situation demands urgent need to find alternative sources of antimicrobial agents. In this context, plant-derived compounds and phytochemicals have gained significant popularity owing to their potential to offer novel treatment strategies to tackle nefarious pathogens[1-4]. The current study focusses on one such plant, *Pedilium murex*, which has shown great promise in early studies for its antibacterial properties against obnoxious disease-causing pathogens.

Pedilium murex is a perennial herb which is resident of various regions and has been used in conventional medicine for a long time. Several reports have shown ethnomedicinal usage of this plant for amelioration of a range of potential ailments, including infections as well as

inflammation. The plant, *Pedilium murex*, harbours a plethora of bioactive compounds, such as alkaloids, flavonoids, and phenolic acids, all of which are reported to contribute to its health benefits. These compounds not only possess significant antioxidant activity but also showcase potent anti-inflammatory properties that are believed to be the reasons for antimicrobial efficacy of the plant. It is due to this diverse potential, that the plant *Pedilium murex* qualifies as an interesting candidate for further investigation and testing its efficacy as antibacterial agent[5-8].

The current study focuses on several bacterial strains including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus*, and *Pseudomonas*. *E. coli* is commonly resides in the gut, however some strains of the bacterium have been reported to cause serious gastrointestinal infections. On the other hand, *S. aureus* is a Gram-positive bacterium and is reported to cause skin infections and more serious conditions such as pneumonia and sepsis, particularly with methicillin-resistant strains (MRSA), which has limited treatment options. The other bacterial strain, *Bacillus* species, is often beneficial but may lead to induction of foodborne illnesses. The other bacteria *Pseudomonas aeruginosa* is a Gram-negative bacterium and notorious for its resistance to conventional antibiotics, making the treatment particularly challenging in case of immunocompromised individuals[9-10].

The antibacterial activity of plant extracts is dependent on which part of the plant is used, and the solvent used for extraction as well as extraction method. Therefore, the current study aimed to evaluate the antibacterial efficacy of different parts of *Pedilium murex*, specifically the leaves, stems, and flowers, against the selected bacterial pathogens, including, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus*, and *Pseudomonas*. The study utilized different solvents such as distilled water, methanol, chloroform, and petroleum ether with an aim to identify the most effective solvent for extraction of bioactive phytochemicals with antibacterial efficacy. It is important to understand the mechanism behind interaction of plant extracts interact with bacterial pathogens in order to develop novel antibacterial treatments. The findings of the current research will enhance our existing knowledge of *Pedilium murex* as well as augment its potential applications in fighting bacterial infections.

Material 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. Specific voucher number was provided for the plant as RUBL21708.

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 grinded 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 diffeernt solvent. Solvents were distilled water (polar), methanol, chloroform (mid polar) and petroleum ether (non-polar). Extraction was done in sonicato. After filteration, solvents were evalpoarted and dry extractes were dissolved in DMSO to make concentartion of 100 µg/ml.

Evaluation of Antibacterial activity:

The antibacterial activity of plant extracts was performed by well diffusion method**.***E. coli, S. aureus, P. 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 ***Pedillum murex*** *against* ***E. coli****:*

The results in Table 1 and Figure 1 showcase antibacterial activity of 100 µg/l of stem, leaf and flower extract of *Pedillum murex* 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 leaf extract followed by stem and flower extract. The highest antibacterial efficacy in case of both leaf and stem extract was obtained when chloroform was used as a solvent. The diameter of inhibition zone was found to be nearly same in all other conditions. The order of diameter of inhibition zone (indicative of antibacterial efficacy of the plant extracts) was as follows:

Leaf extract > Stem extract > Fruits extract

Stem:

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

Leaves:

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

Fruits:

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

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

Plant part	Water	Methanol	Chloroform	Pet ether	Standard
Stem	13	11	15	12	35
Leaves	11	14	23	11	35
Fruits	11	11	11	13	35

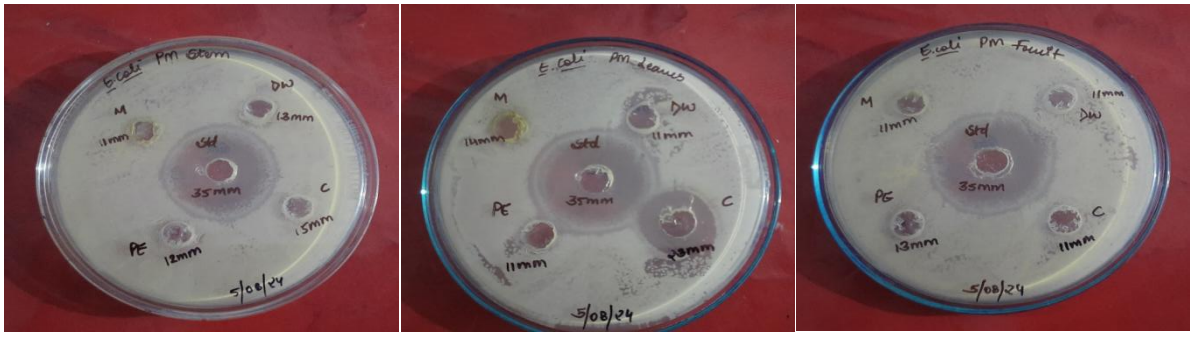

Figure 1: Antibacterial activity of different plant extracts of *Pedillum murex* against *Escherichia coli.*

All the tested extracts demonstrated potent antibacterial efficacy against *E. coli.*, indicating presence of bioactive compounds that serve as potent antibacterial agent in different parts of the plant *Pedillum murex*. Studies have shown presence of several secondary metabolites in *Pedillum murex*, such as alkaloids,

flavonoids, triterpenes, saponins, phenolic compounds and several essential oils, all of which have been reported to showcase potent antibacterial efficacy. However, the highest antibacterial activity against *E. coli* was demonstrated by leaf extract, indicating highest concentration of antibacterial bioactive compounds in the plant leaves. Furthermore, in case of leaf, stem as well as fruits, chloroform extract showcased the highest antibacterial activity, indicative of supremacy of chloroform in efficient extraction of bioactive antibacterial phytochemicals from different plant parts in comparison to other solvents. Several other studies have also demonstrated potent antibacterial efficacy of different plant parts of *Pedillum murex*[11-13].

Antibacterial activity of different parts of ***Pedillum murex*** *against* ***S. aureus****:*

The results in Table 2 and Figure 2 showcase antibacterial activity of 100 µg/l of stem, leaf and flower extract of *Pedillum murex* obtained using different solvents (Distilled water, methanol, chloroform and Pet. Ether) against *S. aureus* The results indicate potent antibacterial efficacy of all the tested extracts against *S. aureus*, with maximum antibacterial efficacy in case of leaf extract followed by nearly same antibacterial activity in stem and flower extract. The highest antibacterial efficacy in case of both leaf and fruit extract was obtained when petroleum ether was used as a solvent. No noticeable antibacterial efficacy was observed in case of chloroform and water extract (stem), methanol and chloroform extract (leaves) and water, methanol and chloroform extract (fruits). The order of diameter of inhibition zone (indicative of antibacterial efficacy of the plant extracts) was as follows:

Leaf extract > Stem extract = Fruits extract

Stem:

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

Leaves:

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

Fruits:

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

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

Plant part	Water	Methanol	Chloroform	Pet ether	Standard
Stem	NA	12	NA	11	39
Leaves	12	NA	NA	22	39
Fruits	NA	NA	NA	12	39

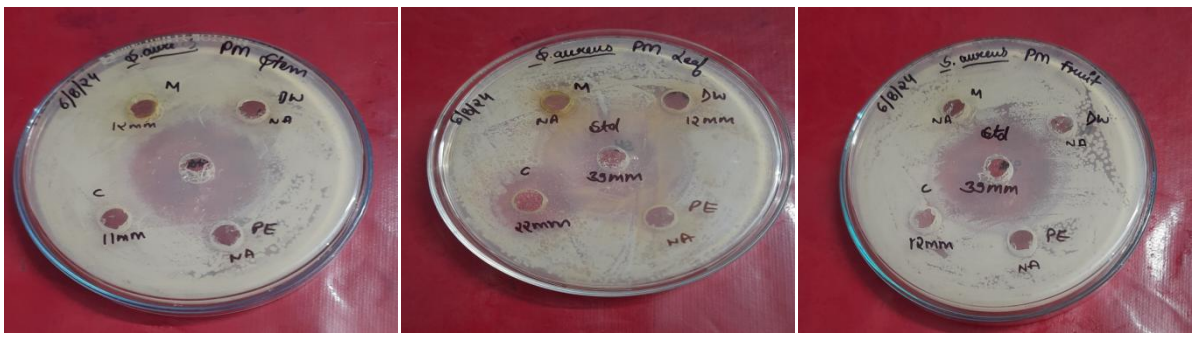

Figure 2: Antibacterial activity of different plant extracts of *Pedillum murex* against *S. aureus.*

In perfect coherence with what was observed in case of *E. coli*, the highest antibacterial efficacy against S. aureus was observed in case of leaf extract followed by nearly similar antibacterial efficacy in case of stem and fruit extracts. However, unlike the gram-negative *E. coli*, which showcased maximum susceptibility to chloroform extracts, the highest susceptibility in case of gram-positive *S. aureus* was observed in case of petroleum ether extracts. Chloroform is moderately polar showcases potency in extraction of alkaloids, triterpenes and lipophilic substances. On the contrary, petroleum ether is non-polar in nature and effective for extraction of non-polar compounds like terpenes, fats and essential oils. The highest antibacterial activity against *S. aureus* being displayed by petroleum ether extracts is indicative of the ability of petroleum ether to selectively extract bioactive antibacterial phytochemicals that are capable of neutralizing the gram-positive bacteria S. aureus but showcase limited antibacterial activity against the gram-negative *E. coli.* Several other studies have also demonstrated potent antibacterial efficacy of different plant parts of *Pedillum murex* against *S. aureus*[14-16].

Antibacterial activity of different parts of ***Pedillum murex*** *against* ***Bacillus subtilis****:* The results in Table 3 and Figure 3 showcase antibacterial activity of 100 µg/l of stem, leaf and flower extract of *Pedillum murex* obtained using different solvents (Distilled water, methanol, chloroform and Pet. Ether) against *Bacillus*.

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

Plant part	Water	Methanol	Chloroform	Pet ether	Standard
Stem	11	NA	13	12	34
Leaves	11	12	12	24	34
Fruits	11	13	11	NA	39

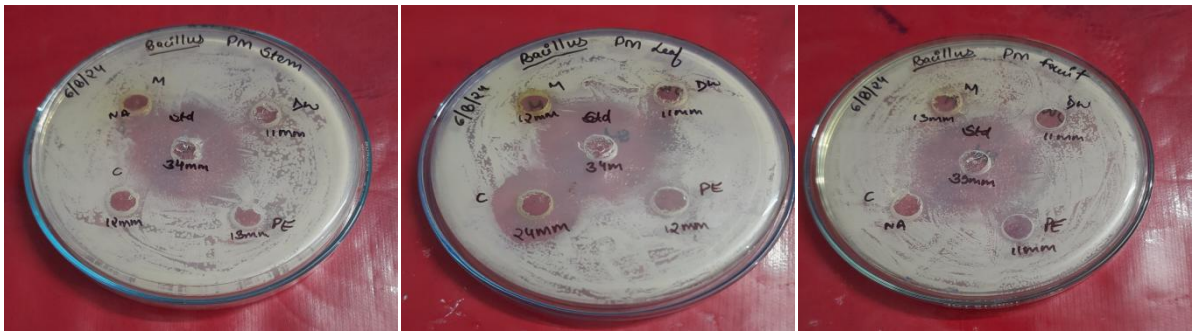

Figure 3: Antibacterial activity of different plant extracts of *Pedillum murex* against *Bacillus.*

The results indicate potent antibacterial efficacy of all the tested extracts against *Bacillus*, with maximum antibacterial efficacy in case of leaf extract followed nearly same antibacterial activity in stem and flower extract. The highest antibacterial efficacy in leaf extract was obtained when petroleum ether was used as a solvent. On the contrary, stem and fruit extract showed highest antibacterial activity when chloroform and methanol were used as solvents respectively. The order of diameter of inhibition zone (indicative of antibacterial efficacy of the plant extracts) was as follows:

Leaf extract > Stem extract = Fruits extract

Stem:

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

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

Exactly similar to what was observed in previous cases, the highest antibacterial efficacy against Bacillus was demonstrated by leaf extracts in comparison to stem and fruit extract. This is in perfect concordance with the previous results, indicative of the highest amount of bioactive antibacterial phytochemicals in plant leaves in comparison to other plants parts such as stem and fruits. Furthermore, highest antibacterial activity was observed in case of petroleum ether extract (leaves) and chloroform extract (stem), indicating supremacy of both petroleum ether and chloroform in extraction of bioactive phytochemicals that showcase potent antibacterial activity against Bacillus. Several other studies have also demonstrated potent antibacterial efficacy of different plant parts of *Pedillum murex* against different strains of *Bacillus*[17-18].

*Antibacterial activity of different parts of **Pedillum murex** against **Pseudomonas aeruginosa**:*

The results in Table 4 and Figure 4 showcase antibacterial activity of 100 µg/l of stem, leaf and flower extract of *Pedillum murex* obtained using different solvents (Distilled water, methanol, chloroform and Pet. Ether) against *Pseudomonas.* The results indicate variable antibacterial efficacy of the tested extracts against *Pseudomonas*, with maximum antibacterial efficacy in case of leaf extract while flower and stem extract did not showcase any antibacterial activity.

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

Plant part	Water	Methanol	Chloroform	Pet ether	Standard
Stem	8	NA	NA	NA	40
Leaves	NA	NA	24	NA	40
Fruits	NA	NA	NA	NA	40

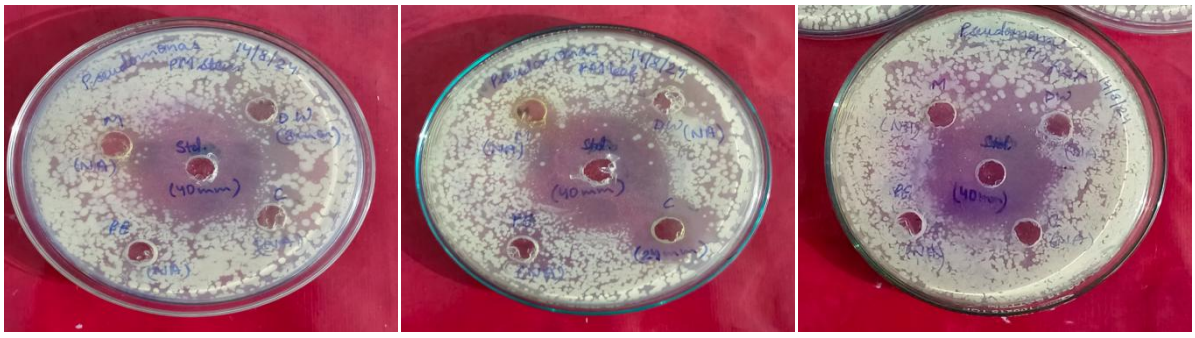

Figure 4: Antibacterial activity of different plant extracts of *Pedillum murex* against *Pseudomonas.*

The leaf extract showcased highest antibacterial efficacy when chloroform was used as a solvent, while no other solvents showed noticeable antibacterial efficacy. Out of the stem and leaf extract, only distilled water stem extract showed slight antibacterial activity whereas all other conditions showed lack of noticeable antibacterial activity. The order of diameter of inhibition zone (indicative of antibacterial efficacy of the plant extracts) was as follows:
Leaf extract > Stem extract = Fruits extract

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

None of the plant extracts using different solvent mixtures displayed potent antibacterial activity against *Pseudomonas.* This may occur due to several reasons, including, ability of *Pseudomonas* to form biofilm which render bacteria impervious to phytochemicals as well as antibiotics. Additionally, *Pseudomonas* possesses low permeability outer membrane, which restricts the entry of phytochemicals. The situation is further exacerbated by presence of drug efflux pumps, which excrete out the antibiotics and phytochemicals. Also, maybe usage of different solvents would be beneficial for extraction of phytochemicals that show antibacterial activity against *Pseudomonas*. Nonetheless, there are studies that showcase potent antibacterial activity of different plant extracts against the bacterium *Pseudomonas aeruginosa*[19-20]**.**

Conclusion

The current study investigated the antibacterial properties of *Pedilium murex* extracts obtained from different plant parts against several bacterial strains, including *E. coli*, *S. aureus*, *Bacillus*, and *Pseudomonas*. The findings of the study showed that the extracts were particularly effective against *E. coli*, *S. aureus*, and *Bacillus*, whereas no noticeable antibacterial activity was observed against *Pseudomonas aeruginosa*. The leaf extracts showcased the highest antibacterial activity especially when chloroform or petroleum ether was used as a solvent. The findings of the study suggests that *Pedilium murex* is a rich storehouse of bioactive compounds that could be utilized for therapeutic purposes. However, the extracts did not show significant antibacterial activity against *Pseudomonas*, showcasing the bacterium's complex resistance mechanisms. This highlights the requirement for further research in order to gain better understanding of the ways in which plant-derived compounds interact with such resistant pathogens. Furthermore, the study emphasizes on the importance of solvent choice in the extraction process of phytochemicals, as solvent greatly affects the efficacy of the antibacterial activity. Overall, the results highlight that *Pedilium murex* holds great promise as a natural source of antibacterial agents, particularly for treatment of common infections. This study opens the frontiers for further investigations into *Pedilium murex* and its bioactive phytochemicals, aiming to reveal the arsenal of bioactive phytochemicals and their antibacterial mechanisms of action as well as potential synergistic effects with conventional antibiotics.

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