

Original Research Article

Antimicrobial Effect of *Passiflora foetida* Leaf ExtractSrijonee Chowdhury¹, Achyuta Kumar¹, Bhaskar Narayan Chaudhuri², Partha Guchhait², Arup Kumar Dawn², Satadal Das^{2*}¹Department of Microbiology, St. Xavier's College (Autonomous), Kolkata, India²Peerless Hospitex Hospital and B.K. Roy Research Centre Ltd., Kolkata, India

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Abstract: Antimicrobial resistance (AMR) is a critical global health concern under the present clinical scenario due to the overuse and misuse of antibiotics in both medical and agricultural sectors. *Passiflora foetida*, commonly known as wild maracuja or stinking passion flower belongs to the Passifloraceae family and is native to tropical and subtropical regions of Asia, Africa, Australia, and America. In this study, we aimed to explore the potential antimicrobial efficacy of *Passiflora foetida* leaf extract against both MDR bacteria and American Type Culture Collection (ATCC) strains for possible combating microbial infections in the future. The MIC value of *Passiflora foetida* extract varied between 5-10 mg/ml in different ATCC and MDR bacteria studied in this experiment. *Passiflora foetida* is also well known in traditional medicine indicating a degree of safety and efficacy, although modern scientific validation is necessary to support its therapeutic applications. Future research should focus on identifying the specific bioactive agent responsible for its antimicrobial effects, optimizing extraction methods to maximize bioavailability, and evaluating its safety and efficacy in animal models and clinical trials. Understanding the antimicrobial properties of *Passiflora foetida* is crucial for developing effective therapeutic strategies against antibiotic-resistant infections. By harnessing the natural antimicrobial potential of this plant, researchers may uncover new treatment options to combat AMR and improve patient outcomes. The findings of this study indicated its possible use as a topical application at present till further study with refined extract may reveal its application in vivo in different diseases with MDR microorganisms.

Keywords: *Passiflora foetida*, antimicrobial agent, MDR bacteria.

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INTRODUCTION

Antimicrobial resistance (AMR) remains a critical global health concern exacerbated by the overuse and misuse of antibiotics in both medical and agricultural sectors. The World Health Organization (WHO) has highlighted AMR as one of the greatest threats to human health in the 21st century, predicting dire consequences if effective countermeasures are not implemented urgently (WHO, 2020). Challenging the efficacy of conventional antibiotics and necessitating the exploration of alternative therapeutic agents. Among these alternatives, natural products derived from plants have emerged as promising candidates due to their diverse chemical composition and historical use in traditional medicine. In recent years, the emergence and spread of multidrug-resistant (MDR) bacteria pose a significant threat to public health worldwide. The limited

effectiveness of conventional antibiotics against these resilient pathogens necessitates exploration into alternative therapeutic agents derived from natural sources. *Passiflora foetida*, commonly known as wild maracuja or stinking passionflower, has garnered attention due to its reported antimicrobial properties. This plant species belongs to the Passifloraceae family. It is native to tropical and subtropical regions and is widely distributed across continents such as Asia, Africa, Australia, and the Americas. This plant is characterized by its vigorous growth habit, climbing vine structure, and distinctive flowers and fruits. Beyond its ornamental and culinary uses, this plant has a rich history in traditional medicine, where it has been employed to treat various ailments, including infections. The potential antimicrobial efficacy of *Passiflora foetida* leaf extract against both MDR bacteria and American Type Culture Collection (ATCC) strains presents an intriguing avenue

*Corresponding Author: Satadal Das

Peerless Hospitex Hospital and B.K. Roy Research Centre Ltd., Kolkata, India

for research and development in combating microbial infections.

Natural products, especially those derived from medicinal plants, have historically served as reservoirs of bioactive compounds with therapeutic potential against various ailments, including infectious diseases. *Passiflora foetida*, known for its diverse pharmacological properties, has been traditionally used in folk medicine for treating conditions ranging from gastrointestinal disorders to microbial infections [2]. The antimicrobial activity of *Passiflora foetida* is attributed to its complex phytochemical profile, which includes flavonoids, alkaloids, phenolic compounds, and essential oils. These bioactive constituents have demonstrated various biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects [3]. Specifically, their ability to disrupt bacterial cell membranes, inhibit essential enzymatic processes, and interfere with microbial biofilm formation makes *Passiflora foetida* extracts particularly intriguing for combating antibiotic-resistant bacteria.

Several studies have demonstrated promising results regarding the antimicrobial activity of *Passiflora foetida* extracts. For instance, a study investigated the inhibitory effects of *Passiflora foetida* leaf extract against a panel of MDR bacterial strains, including Methicillin-resistant *Staphylococcus aureus* (MRSA) and Extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae. The researchers reported significant antimicrobial activity, with minimum inhibitory concentrations (MICs) demonstrating potent bacteriostatic or bactericidal effects against the tested strains.

Previous studies have demonstrated the antimicrobial potential of *Passiflora foetida* extracts against a spectrum of bacterial species, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. These findings underscore the need for further investigation into its activity against clinically relevant MDR strains, which present a formidable challenge in healthcare settings. By elucidating the mechanisms underlying its antimicrobial action and identifying active compounds responsible for inhibitory effects, researchers can potentially harness *Passiflora foetida* as a novel therapeutic agent or adjunct to conventional antibiotics [1].

The mechanisms underlying the antimicrobial activity of *Passiflora foetida* extracts are multifaceted. Flavonoids and phenolic compounds within the extracts have been shown to disrupt bacterial cell membrane integrity, leading to leakage of cellular contents and eventual cell death. Alkaloids present in *Passiflora foetida* extracts may interfere with microbial protein synthesis or disrupt metabolic pathways essential for bacterial survival. Furthermore, the synergistic interactions among these phytochemicals can enhance

overall antimicrobial efficacy, potentially overcoming bacterial resistance mechanisms.

Passiflora foetida contains several organic compounds that have demonstrated antimicrobial activity, though specific resistance mechanisms among pathogens can vary. Two notable organic compounds found in *Passiflora foetida* known for their antimicrobial properties are vitexin and quercetin.

Quercetin (Fig. 1) is a flavonoid compound widely distributed in plants, including *Passiflora foetida*. It is known for its antioxidant, anti-inflammatory, and antimicrobial activities. Quercetin has been studied for its effectiveness against a variety of bacteria and fungi. Quercetin can destabilize bacterial cell membranes, leading to leakage of cellular contents and eventual cell death.

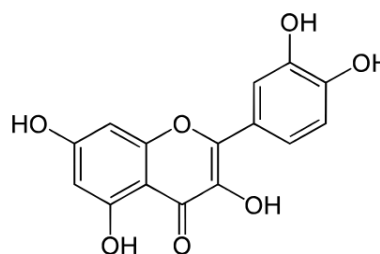


Fig. 1: Quercetin

Vitexin (Fig. 2) is a specific flavonoid glycoside. It is known for its diverse biological activities and potential health benefits. Vitexin exhibits strong antioxidant activity, helping to scavenge free radicals and reduce oxidative stress in the body. Vitexin has antimicrobial properties against various pathogens, including bacteria and fungi. This makes it valuable in traditional medicine practices where *Passiflora foetida* is used to treat infections. Vitexin has been investigated for its neuroprotective effects, including potential benefits in conditions related to neurodegeneration and cognitive function. Vitexin has potential to inhibit cancer cell growth and inducing apoptosis (programmed cell death) in certain types of cancer cells.

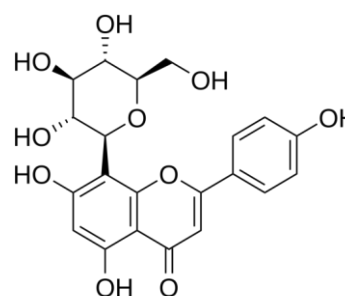


Fig. 2: Vitexin

It's important to note that while vitexin, quercetin and other organic compounds in *Passiflora foetida* show promising antimicrobial activity, the development of resistance among bacterial pathogens

remains a significant concern in antimicrobial therapy. Ongoing research is necessary to understand the full spectrum of antimicrobial activities of vitexin, quercetin and other compounds from *Passiflora foetida*, as well as strategies to mitigate or overcome potential resistance mechanisms in microbial populations.

This research aims to evaluate the antimicrobial efficacy of *Passiflora foetida* leaf extract against both multidrug-resistant bacterial strains and ATCC reference strains. Specific objectives include isolating and characterizing multidrug-resistant bacterial strains from clinical samples, extracting bioactive compounds from *Passiflora foetida* leaves using suitable extraction techniques, assessing the antimicrobial activity of *Passiflora foetida* leaf extract through well-established microbiological assays, investigating the mechanisms of action underlying the observed antimicrobial effects, including potential synergistic interactions with antibiotics, comparing the efficacy of *Passiflora foetida* extract against ATCC strains with known susceptibility profiles.

MATERIALS AND METHODS

Under this research, we attempted to study the antimicrobial properties of *Passiflora foetida* against some specific multidrug resistant bacterial species namely *Staphylococcus aureus* (ATCC 29213), Methicillin Resistant *Staphylococcus aureus*, *Escherichia coli* (ATCC 25922), *Escherichia coli* (MDR), *Klebsiella spp.* and *Pseudomonas spp.* Firstly, *Passiflora foetida* leaves were procured and crushed into powder. 200mg of the powdered leaf was weighed on an analytical balance and then suspended in 5ml of ethanol (0.04gm/ml) for 48 hrs. The suspension was vortexed repeatedly to ensure thorough dissolution of the leaf extract in ethanol. The minimum inhibitory concentration (MIC) of the leaf extract, for each bacterial species, was assayed in a 96-welled microtitre plate.

Bacterial isolates were dissolved in normal saline (NS) to maintain pH and tonicity, and to prevent lysis of the viable bacterial cells. The bacterial suspensions were maintained at 0.5 McFarland opacity using DensiCHEK.

100µl of Mueller-Hinton broth was pipetted into each of the 96 wells of the titer plate. 100µl of the leaf extract was added to the first well which was serially diluted till the eighth well, progressively diminishing the concentration of the leaf extract. The surplus 100µl of mixture left at the end of the column was discarded. Similarly, 100µl of ethanol was added to the adjacent well and serially diluted across the column as a control for absorbance measurement. In total, six sets of leaf extract and ethanolic control were prepared in 12 columns for six bacterial isolates respectively. 10µl of each bacterial isolate was added to its respective set of leaf extract and control and mixed thoroughly. At 0hr, absorbance was first measured at 620nm. After overnight incubation at 37°C, absorbance was rerecorded at 620nm at 24hrs. The difference of the absorbance was plotted on the Y-axis with the respective concentrations on the X-axis and MIC values were thus noted and recorded from the representative graphs. Independent antimicrobial effect of leaf extract was plated on lawn culture of *Pseudomonas spp.* Antibiotic synergy test of the leaf extract was observed in association with Antibiotic drugs, Meropenem and Polymyxin B against *E. coli* (MDR) and *Pseudomonas spp.*

RESULTS

The minimum inhibitory concentration (MIC) of the leaf extract (Fig.3-8) was observed to be as follows: *Staphylococcus aureus* (ATCC 25923) (MIC: 10mg/ml), Methicillin Resistant *Staphylococcus aureus* (MIC: 5mg/ml), *Escherichia coli* (ATCC 25922) (MIC: 5mg/ml), *Escherichia coli* (MDR)(MIC: 5mg/ml), *Klebsiella spp.*(MDR)(MIC: 5mg/ml). and *Pseudomonas spp.* (MDR) (MIC: 5mg/ml).

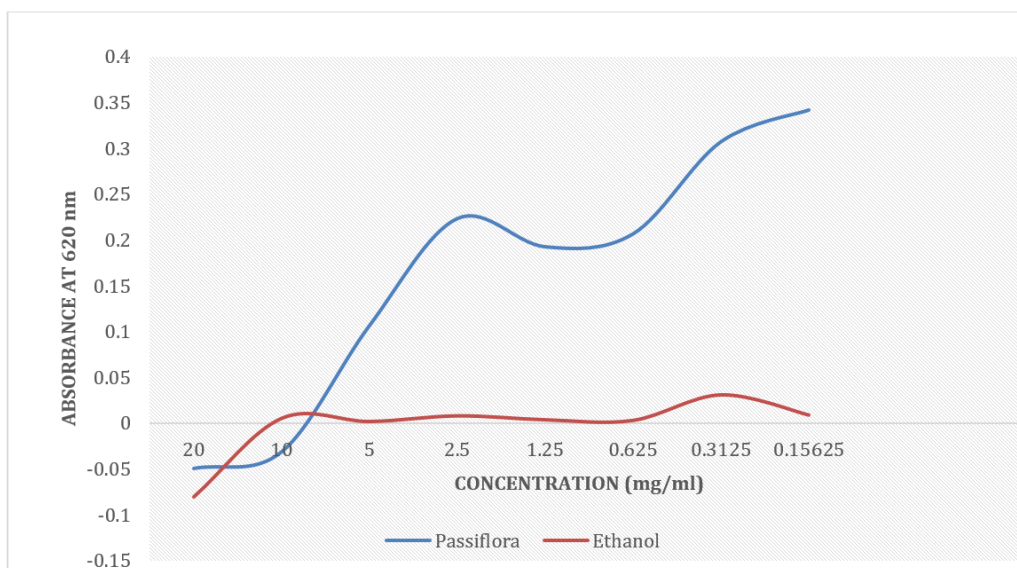


Fig. 3: Antimicrobial activity of *Passiflora* extract against *Staphylococcus aureus* ATCC 25923, MIC value 10 mg/ml

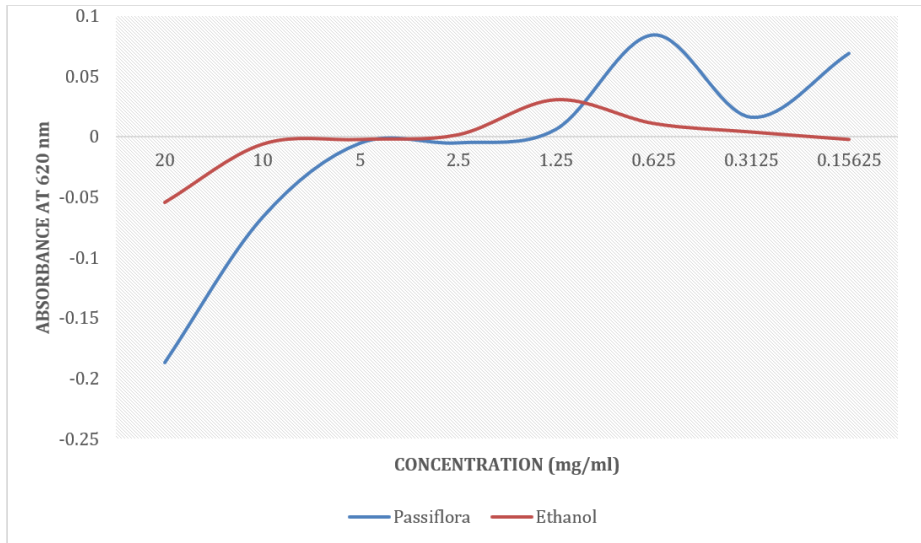


Fig. 4: Antimicrobial activity of *Passiflora* extract against MRSA, MIC value 5 mg/ml

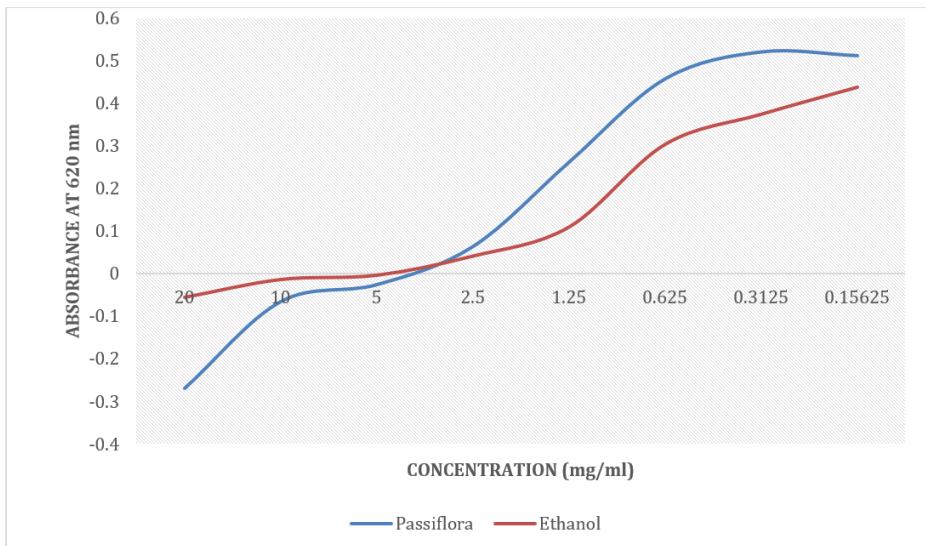


Fig. 5: Antimicrobial activity of *Passiflora* extract against *E. coli* ATCC 25922, MIC value 5 mg/ml

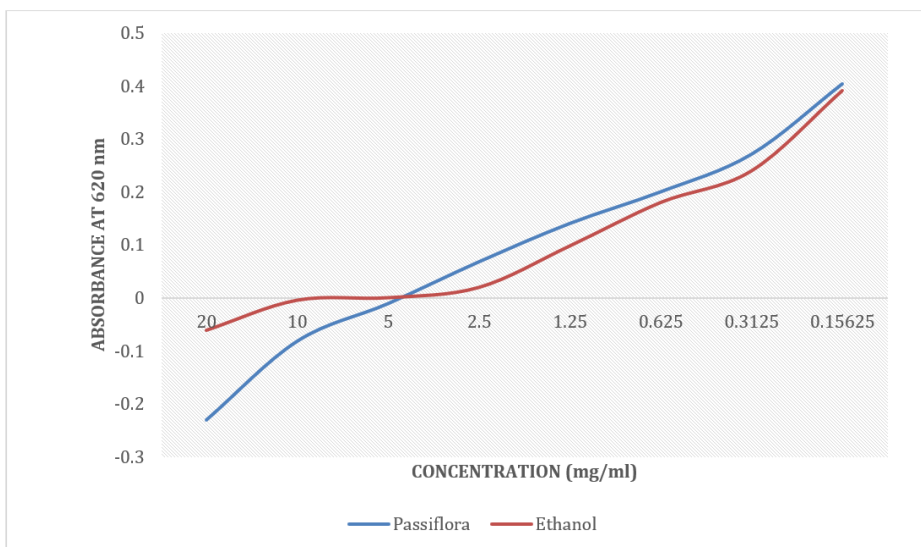


Fig. 6: Antimicrobial activity of *Passiflora* extract against *E. coli* MDR, MIC value 5 mg/ml

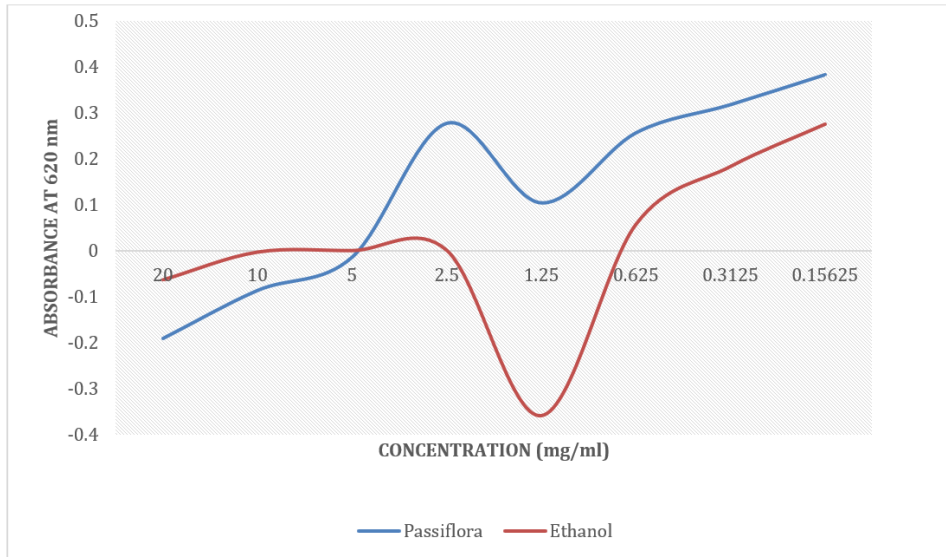


Fig. 7: Antimicrobial activity of *Passiflora* extract against *Klebsiella* MDR, MIC value 5 mg/ml

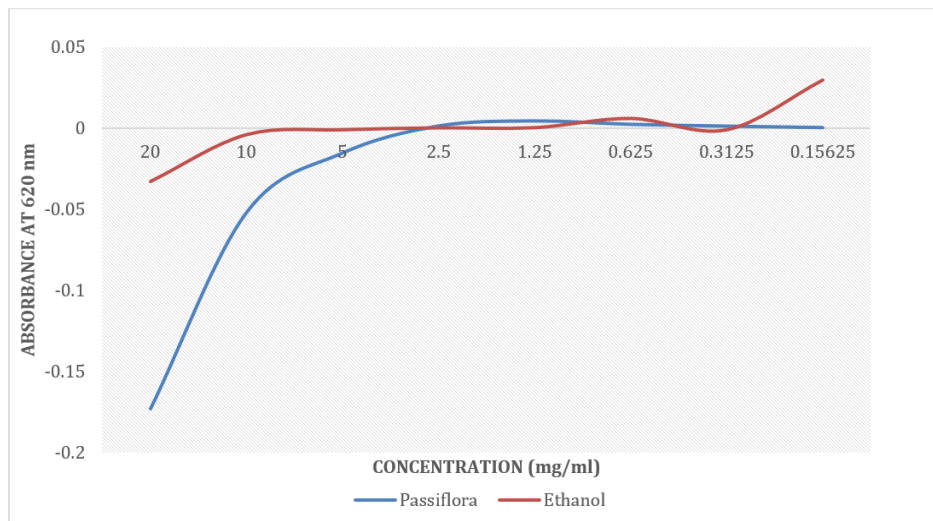


Fig. 8: Antimicrobial activity of *Passiflora* extract against *Pseudomonas* MDR, MIC value 5 mg/ml

Antimicrobial resistance of the leaf extract (20mg/ml) was prevalent when plated directly on Mueller-Hinton Agar plate by disk diffusion method using sterile discs pre-soaked in the leaf extract. In

association with Meropenem and Polymyxin B the *Passiflora foetida* leaf extract showed antagonistic effects against *Pseudomonas* spp. and *E. coli* (MDR).

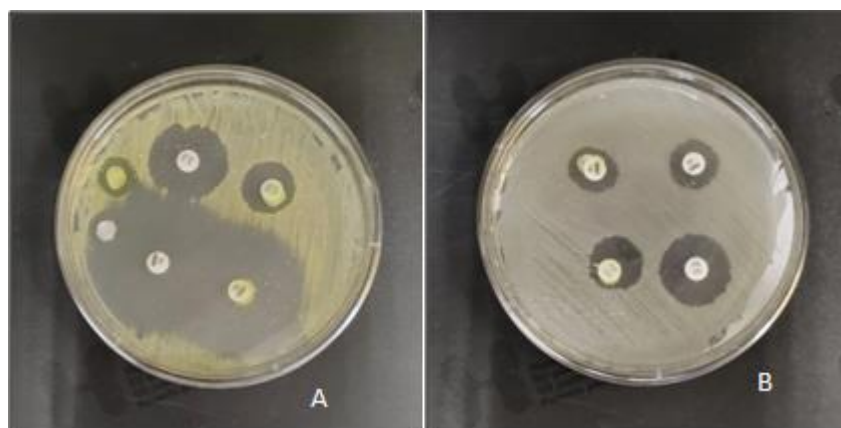


Fig. 9: Antagonistic action of *Passiflora* extract on *Pseudomonas* (A) and *Staphylococcus* (B)

DISCUSSION

Many biological activities of *Passiflora* have been demonstrated in last two decades which include anti-inflammatory, antiviral, antibacterial, antioxidant, analgesic, diuretic action etc. [6-9]. The bioactive chemicals present in the extract are alkaloids, saponins, flavonoids, essential oils, carotenoids etc. [10]. In this work, we observed that the extract has got antimicrobial activities against both ATCC and MDR strains including MRSA. The antimicrobial activity of this extract is also well known in agriculture. Antibacterial activities of this plant extract was also observed by many workers [11, 12]. However, Vasic *et al.*, [13] stated that the antibacterial activity was not related to these phytoconstituents. Johnson *et al.*, [14] also observed antimicrobial action of this plant extract on *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Aeromonas spp.*, *Serratia* etc.

CONCLUSION

The MIC value of *Passiflora foetida* leaf extract varied between 5-10 mg/ml in different ATCC and MDR bacteria. Thus *Passiflora foetida* - a well known in traditional medicine with safety and efficacy can be used as antimicrobial agent in infectious diseases of human beings.

Conflict of Interests: No conflict of interest was declared.

Source of Funding: There has been no source of funding.

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Author's Contribution

Dr. Satadal Das conceived and designed the study and Srijonee Chowdhury and Achyuta Kumar played pivotal roles in performing the experiments. Dr. Bhaskar Narayan Chaudhuri and Arup Kumar Dawn contributed significantly by providing essential tools and lab equipments crucial for executing the experiments. Analysis and interpretation of experimental results were collectively undertaken by Dr. Satadal Das, Srijonee Chowdhury, and Achyuta Kumar. Dr. Satadal Das played a crucial role in critically revising the manuscript, initially drafted by Srijonee Chowdhury and Achyuta Kumar.

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