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Antibacterial Activity of Padikara Parpam against ESBL Producing *Escherichia coli* and *Klebsiella pneumoniae*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To evaluate the antibacterial activity of Padikara Parpam against Extended-Spectrum Beta-Lactamase (ESBL) producing *Escherichia Coli* and *Klebsiella Pneumoniae* using agar well diffusion method. To identify ESBL producing bacteria by phenotypic confirmatory test using disk diffusion method.
Study Design: Analysis of Antibacterial activity of Padikara Parpam using agar well diffusion method.
Place and Duration of Study: Central Research Laboratory, Meenakshi Academy of higher Education and Research, Chennai, between June 2021 and November 2021.
Methodology: Clinical isolates of ESBL were isolated by subculture into MacConkey agar and was identified by phenotypic confirmatory test. Padikara parpam's antibacterial activity was evaluated using the Agar well diffusion method at different concentrations of 0.5 %, 1 %, 1.5 %, and 2 % drugs. 30 µg Cefotaxime and 30 µg amoxicillin-clavulanic acid disk were used as controls to standardize the antibacterial activity test and to identify the ESBL by phenotypic confirmatory test.
Results: In this study, Padikara parpam at various doses of 0.5 %, 1 %, 1.5 %, and 2 %, revealed significant antibacterial efficacy against ESBL producing bacteria. Padikara parpam was more

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active against ESBL *Escherichia coli* than ESBL *Klebsiella pneumoniae.* As a result, it may be recommended as an antibacterial agent against ESBL.
Conclusion: Our findings suggest that Siddha Herbo mineral formulations of padikara parpam hold phenomenal antimicrobial activity against ESBL producing bacteria. Based on our findings, the drug may be prescribed successfully for urinary tract infections, which is caused by ESBL producing bacteria.

Keywords: Padikara Parpam; ESBL; Escherichia coli; Klebsiella pneumoniae; diffusion method.

1. INTRODUCTION

Microbiology has proven to be one of biology's most important sciences, allowing researchers to figure out how specific organisms cause diseases, find therapies for those ailments, and even exploit bacteria for industrial reasons, among other things. Microbes that are clinically important are pathogens that cause a variety of infectious illnesses. The most common microorganism is bacteria. ESBLs (extended-spectrum beta-lactamases) are enzymes or chemicals produced by bacteria and other organisms. Antibiotics have a greater difficulty treating bacterial infections because of these enzymes. *Escherichia coli* and *Klebsiella pneumoniae* are the two most common bacteria that produce ESBLs [1].

Infectious diseases are a major cause of death worldwide, with tropical regions responsible for more than half of all deaths [2]. Pathogens are clinically significant and are considered to be primary contributors of hospital-acquired infection. Furthermore, they are common infection sources in the community. Antibiotics are used to treat a range of infections [3-8]. Some plant extracts and photochemical have been shown to have antimicrobial characteristics, which can be highly valuable in medical treatments. In recent years, a number of researches in various nations have been conducted to demonstrate its efficacy [2].

One of India's greatest cultural contributions is the Siddha system of medicine. In the Siddha system of medicine, medications made from metal and mineral compounds, such as Parpam, Chendooram, and Chunnam, are used to treat most chronic ailments. In Indian medicine, these siddha compositions are used to treat a variety of diseases. Many of the remedies described in the siddha system of medicine proved to be an effective therapy against various diseases. They are used to protect human life resources from natural disasters, and many of the discoveries have been confirmed by modern scientific investigation [9].

In siddha medicine, Padikara parpam is used to treat urinary retention, painful micturition, inflammation of the urinogenital organ, hematuria and urinary blockage, gonorrhea, stomatitis, menorrhagia [10]. It is mostly used to treat water in domestic applications, and widely used as a disinfectant and antiseptic for therapeutic purposes**.**

2. MATERIALS AND METHODS

2.1 Materials

PadikaraParpam, ESBL producing bacteria - *Escherichia coli, Klebsiella pneumoniae*, Controls– Cefotaxime (30 µg) and amoxicillin-clavulanic acid (30µg). Mueller Hinton Agar (MHA).

2.2 Methods

2.2.1 Collection of material

Padikara Parpam was procured from IMPCOPS pharmaceuticals, Chennai. In traditional Indian medical care, these commercially available medicines are used to treat a variety of diseases. In siddha medicine, they are usually given in doses of 200 -300 mg with ghee or honey, twice a day after food for diseases including ECZEMA, Chronic disease, Oedema, Swelling, Dysuria, etc.

2.2.2 Collection of bacterial isolates

For this experiment, clinical isolates were collected from clinical laboratory (VRR Diagnostics, T.Nagar, chennai). Antibacterial susceptibility testing is evaluated using these isolated clinical samples. Stock cultures are stored in 20% glycerol at -70^{o}C for long-term storage, whereas working cultures are kept on Nutrient agar (NA) slants at 4^{o}C. The collected

strains were transported to MAHER's Central Research Laboratory in Chennai.

2.2.3 Identification of bacterial isolates

Clinically isolated cultures are inoculated into MacConkey agar. After inoculation, the colonies are incubated for 24 hours at 37°C. After incubation, the isolated colonies were identified by Gram's stain and standard biochemical tests. Following incubation, ESBL producing bacteria were identified by using Phenotypic Confirmatory Test.

2.2.4 Preparation of the inoculums

Clinically isolated bacterial strains of ESBL producing bacteria are inoculated in peptone water and incubated for 4 hours at 37°C. 0.5 McFarland standards ($1.5x10^8$cfu/ml) are used to adjust the inoculum.

2.2.5 Preparation of the drug solution

Dissolve 0.1 gm of the drug in 1 mL of distilled water to make the 1 % drug solution. For this study, different concentrations of 0.5 %, 1 %, 1.5 %, and 2 % drugs were prepared.

2.2.6 Antimicrobial screening

The antibacterial activity was tested using the agar well diffusion technique. The zone of inhibition was used to analyze the outcomes. ESBL producing bacteria were identified by Phenotypic Confirmatory Test using Kirby Bauer disc diffusion method. ESBL- producing *Escherichia coli* and *Klebsiella pneumoniae* are inoculated in peptone water and incubated for 4 hours at 37°C. About, 0.5 McFarland standards ($1.5x10^8$cfu/ml) are used to adjust the inoculum. Using sterile Swabs, the bacteria are spread on Mueller Hinton Agar plates. Wells are punched in the agar plates with the use of a sterile tool. In the wells, 50 µl of varying concentrations of prepared drugs were injected by agar well diffusion method. Few antibiotics, such as 30 µg Cefotaxime and 30 µg amoxicillin-clavulanic acid disk were used as a control and were used to identify the ESBL producing bacteria by Phenotypic Confirmatory Test using Kirby Bauer disc diffusion method. These culture plates were incubated at 37°C for 24 hours. At the end of experiment, the zones of inhibition were measured [11].

2.2.7 Analysis of zone of inhibition

The inhibition of zone size is estimated in millimeters (mm). Zone inhibition is interpreted as a lack of activity when it is absent. If the zone of inhibition is less than 7 mm, the activity is considered as resistant. If it is between 8 and 10 mm, the activity is considered as intermediate. And if it is greater than 11 mm, the activity is considered as sensitive [3]. 0.5 % - 50 mg/ mL of the drug, 1 % - 100 mg/ mL of the drug, 1.5 % - 150 mg/ mL of the drug, 2 % - 200 mg/ mL of the drug, CTX 30 - 30 µg of cefoxitin, AMP-C 30 - 30 µg of amoxicillin-clavulanic acid.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Isolation and identification of bacterial isolates

Clinically isolated bacterial samples of ESBL were sub-cultured on MacConkey agar. MacConkey agar was used to isolate the bacterial strains. Standard microbiological tests were utilized to identify the isolates, including Gram's stain and conventional biochemical assays include Indole test, Citrate test, Triple Sugar Iron test, Urease test and Mannitol Motility Medium test. As shown in Fig. 1, bacterial strains were isolated and identified as *Escherichia coli and Klebsiella pneumoniae.*

3.1.2 Antibacterial activity of Padikara parpam

In this study, Padikara Parpam exhibit antibacterial activity against ESBL producing bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*. 30 µg of Cefotaxime and 30 µg of amoxicillin- clavulanic acid diskswere used to identify the ESBL producing bacteria byphenotypic confirmatory test (Disk diffusion method). Various concentration of 0.5 %, 1.0 %, 1.5 % and 2.0 % drug were used for Agar well diffusion method to evaluate the antibacterial activity. Different concentration of drugs and disk of controls were used, as shown in Fig. 2.

3.1.3 Zone of Inhibition

The zone of inhibition was shown in Fig. 2. Every concentration of prepared drug exhibit antibacterial activity against ESBL producing *Escherichia Coli* and *Klebsiella Pneumoniae.* The antibacterial activity of Padikara parpam

was evaluated by zone of inhibitionfrom Agar well diffusion method.Controls of 30 µg of Cefotaxime and 30µg of amoxicillin-clavulanic acid disk were confirmed the ESBL producing bacteria by phenotypic confirmatory test (Disk diffusion method).The zone of inhibition was determined, as shown in Table 1. The graphical representation of zone of inhibition was shown in Fig. 3.

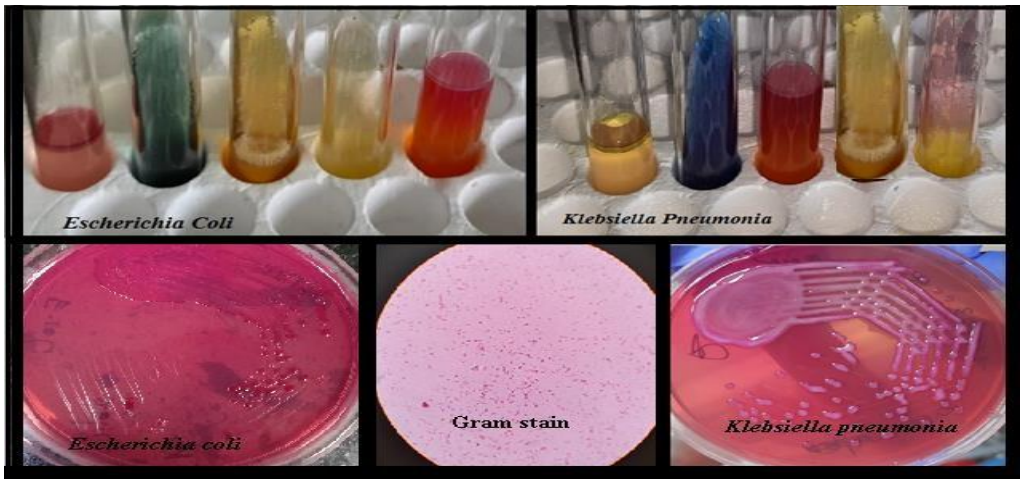

Fig. 1. Isolation and identification of *Escherichia Coli* and *Klebsiella Pneumonia*

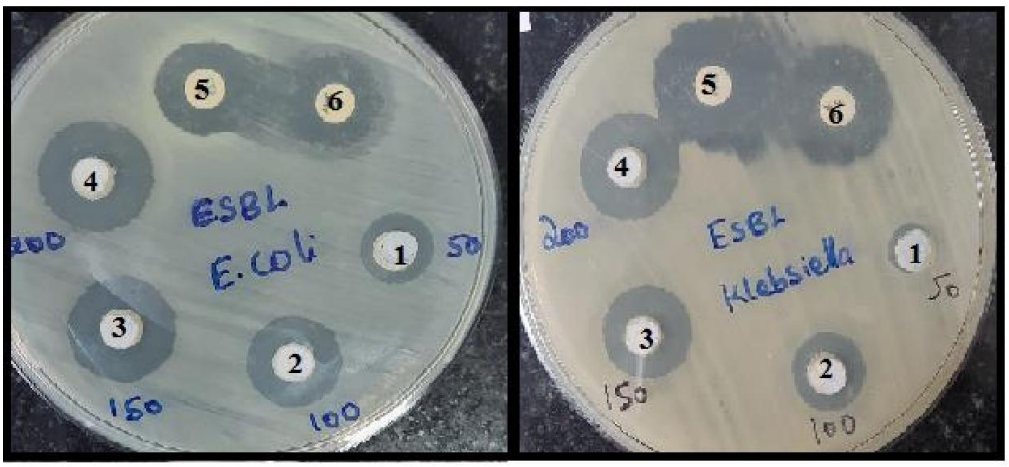

Fig. 2. Antibacterial activity of Padikara Parpam against ESBL producing *Escherichia coli* and *Klebsiella pneumoniae*

1. *0.5 % - 50 mg/ mL of the drug*
2. *1.0 % - 100 mg/ mL of the drug*
3. *1.5 % - 150 mg/ mL of the drug*
4. *2.0 % - 200 mg/ mL of the drug*
5. *CTX 30 - 30 µg of Cefotaxime*
6. *AMP-C 30 - 30µg of amoxicillin-clavulanic acid*

Table 1. The zone of inhibition

Zone Ofinhibition	0.5 %	1.0 %	1.5 %	2.0%	Cefotaxime 30 µg (CTX 30)	Amoxicillin clavulanic acid 30 µg (AMC 30)
Escherichia Coli	14 mm	17 mm	19 mm	22 mm	16 mm	16 mm
Klebsiella Pneumoniae	10 mm	14 mm	17 mm	20 mm	20 mm	21 mm

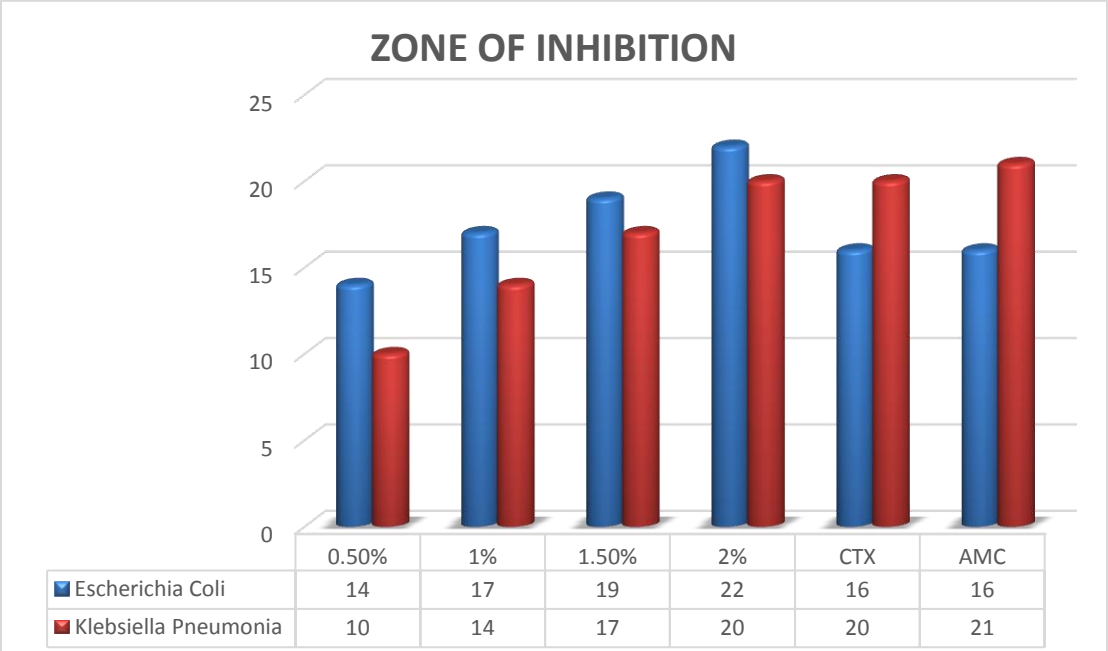

Fig. 3. The graphical representation of zone of inhibition

3.2 Discussion

Padikara Parpam is a drug that contains necessary factors possessing antimicrobial activity against ESBL producing bacteria. The present study is an attempt to unveil the medicinal properties of Padikara Parpam, with special reference to its antibacterial activity. The study is a pointer to highlight the use of siddha medicinal preparation, “Padikara Parpam” as a treatment therapy, based on the present observations as well as the availability of the existing evidences. Only a few studies have used the Kirby and Bauer methods to investigate synergism. Siddha also has a wide spectrum of biological properties [4].

The results of the disc diffusion experiment revealed that there has been an increasing effect on bacterial growth. Our findings suggest that Siddha Herbo mineral formulations of Padikara Parpam have significant antimicrobial activity against ESBL producing bacteria. The drug can be prescribed successfully for infection which is caused by ESBL producing bacteria. As a result, these drug formulations can be utilized to prevent or control enteric bacterial illness also [5]. More clinical trials are required to enhance the success rate of “Padikara Parpam” in the treatment of urinary tract infection [6]. Extensive findings available on herbo–mineral drugs confirmed the potency of the drugs as a promising candidate among the various ethnic groups, viz., Vaidyas, Hakims and ayurvedic practitioners for cure of variety of ailments. As evidence to the above quote, our study forms a pointer to highlight the efficacy of Padikara Parpam as potent antibacterial agent against ESBL producing bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*.

4. CONCLUSION

In conclusion, the study is an evidence of the antibacterial potential of the Siddha drug, “Padikara Parpam” on ESBL- producing bacterial strains. Our previous studies have highlighted the potential of various Parpams towards the pathogenic bacterial types and confirmed the efficiency of Padikara Parpam to a significant extent. Furthermore studies are required to unravel the active compounds that would define the underlying mechanism behind the antibacterial activity of Padikara Parpam.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement

of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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