ANTIBACTERIAL ACTIVITY OF PADIKARA PARPAM AGAINST ESBL PRODUCING *Escherichia Coli* AND *Klebsiella Pneumonia*

ABSTRACT

The Siddha system of medicine is one of the India's greatest traditional legacies. Padikara Parpam is a historical Siddha medicine used to treat urinary infections such as urine retention, painful dysuria, urinogenital organ irritation and haematurea. Extended-Spectrum Beta-Lactamase (ESBL)-producing bacteria, such as *Escherichia coli* and *Klebsiella pneumonia*, cause many dangerous illnesses, including serious urinary tract infections. The purpose of this investigation was to determine the antibacterial activity of Padikara Parpam against ESBL producing bacteria such as *E. coli, K. pneumonia.* ESBL was confirmed using the Phenotypic Confirmatory Disc Diffusion Test (PCDDT) and the Double Disk Synergy Test. Padikara Parpam's antibacterial activity were evaluated using the disc diffusion method at different concentrations of drugs (0.5 %, 1 %, 1.5 %, and 2 %). About, 30 µg cefoxitin and 30 µg amoxicillin-clavulanic acid were used as controls to standardize the antibacterial activity test. Padikara Parpam, at various doses of 0.5%, 1%, 1.5%, and 2%, showed promising antibacterial efficacy against ESBL producing bacteria. Padikara Parpam was discovered to be effective against *E. coli* and *K. pneumonia* isolates, which produce ESBL. Hence, it may be recommended as an antibacterial agent against ESBL.

Keywords: Padikara Parpam, ESBL producing bacteria - *Escherichia coli, Klebsiella pneumonia*, cefoxitin, amoxicillin-clavulanic acid.

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 (Dharmaratne et al., 2018).

Infectious diseases are the major cause of death worldwide, with tropical regions responsible for more than half of all deaths. 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. 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 (Princeet al., 2016).

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. They are used to protect human life resources from natural disasters, and many of the discoveries have been confirmed by modern scientific investigation (Revathi et al., 2018).

In siddha medicine, Padikara parpamis used to treat urinary retention, painful micturition, inflammation of the urinogenital organs, hematuria and urinary blockage, gonorrhea, stomatitis, and menorrhagia. It is mostly used to treat water in domestic applications. It is used as a disinfectant and antiseptic for therapeutic purposes (Saravanasingh et al., 2021).

MATERIALS AND METHODS

Materials:

Padikara Parpam, ESBL producing bacteria - Escherichia coli, Klebsiella pneumonia, Controls – cefoxitin (30 mcg) and amoxicillin-clavulanic acid (30mcg). Mueller Hinton Agar (MHA).

Methods:

Collection of material:

IMPCOPS pharmaceuticals in Chennai provide authenticated Padikara Parpamproducts. In traditional Indian medical care, these commercially available medicines are used to treat a variety of diseases. They're usually given in doses of 100-200 mg per day and should be taken with a suitable supplement. For further processing, the products are shipped to MAHER's Central Laboratory in Chennai.

Collection of bacterial isolates:

For this experiment, clinical isolates are collected from clinical laboratories. 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. For further processing, the strains are transferred to MAHER's Central Laboratory in Chennai.

Identification of bacterial isolates:

Clinically isolated cultures are inoculated into MacConkey agar. After inoculation, the colonies are incubated for 24 hours at 37°C. Isolated colonies are tested for Gram's stain and standard biochemical tests after incubation. Following incubation, these tests are utilised to identify the ESBL producing bacteria by using Phenotypic Confirmatory Disc Diffusion Test (PCDDT) and the Double Disk Synergy Test.

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.

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.

Antimicrobial screening:

The antibacterial activity is tested using the agar well diffusion technique. The zone of inhibition is used to analyze the outcomes. The disc diffusion test is performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2020, 30th edition). ESBL-producing *Escherichia coli* and *Klebsiella pneumonia* are inoculated in peptone water and incubated for 4 hours at 37°C. The inoculum is adjusted using 0.5 McFarland standards (1.5x108cfu/ml). 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. Few antibiotics, such as 30 mcg cefoxitin and 30 mcg amoxicillin-clavulanic acid, are used as a control. T these inoculated culture plates are incubated at 37°C for 24 hours. At the end of experiment, the zones of inhibition are measured (Shobha et al., 2014).

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 (Suvetha et al., 2018). **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

RESULTS AND DISCUSSION

Clinically isolated bacterial samples 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 Figure 1, bacterial strains were isolated and identified as *E. coli* and *K. pneumonia.*

Padikara Parpam has been found to exhibit antibacterial activity against ESBL producing bacteria such as *E. coli* and *K.pneumonia*. About 30 µg of cefoxitin and 30 µg of amoxicillin-clavulanic acid were used to identify the ESBL producing bacteria by using Double Disk Synergy Test. The zone of inhibition was determined, as shown in Figure 2. Padikara Parpam is a drug that contains necessary factors possessing antimicrobial activity against ESBL producing bacteria. To summarize, the statement made in the siddha system of medicine has been scientifically proven. The purpose of antimicrobial activity was to look at the past, present and future of medicinal plants in order to recommend. Only a few studies have used the Kirby and Bauer methods to investigate synergism. Siddha also has a wide spectrum of biological properties (Purushotham, 2010).

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 a variety of conditions in UTI 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 (Michael, 2011). To enhance clinical success in the control and treatment of urinary tract infections, more literature formulations must be developed (Rajalakshmi, 2016). Furthermore studies are required to unravel the active compounds that would define the underlying mechanism of the antibacterial activity of Padikara Parpam.

The drug can be prescribed successfully for a variety of conditions in UTI 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 (Michael, 2011). To enhance clinical success in the control and treatment of urinary tract infections, more literature formulations must be developed (Rajalakshmi, 2016). Furthermore studies are required to unravel the active compounds that would

define the underlying mechanism of the antibacterial activity of Padikara Parpam.

For many decades, Siddha drugs find greater application against various ailments (Uma et al., 2012; Handral et al., 2012). Nevertheless, the lead molecule responsible for the bio-pharmacological action remains unknown, which urge the need of exploring the active principle through biochemical and molecular biological techniques. Moreover, animal and human trials have to be carried out to understand the pharmacodynamics ad bioavailability of the drugs.

Conclusion

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 *E. coli* and *K. pneumonia*.

NOTE:

The study highlights the efficacy of "siddha medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

REFERENCES

1. Dharmaratne, M. P. J., Manoraj, A., Thevanesam, V., Ekanayake, A., Kumar, N. S., Liyanapathirana, V., & Bandara, B. R. (2018). Terminalia bellirica fruit extracts: in-vitro antibacterial activity against selected multidrug-resistant bacteria, radical scavenging activity and cytotoxicity study on BHK-21 cells. *BMC complementary and alternative medicine*, *18*(1), 1-12.
2. Prince, C. P., & Gopal, V. (2016). Antibacterial activity of Siddha drugs, MathanThailam and Punga Thailam against clinically isolated MRSA strains. *Journal of Pharma Research*, *5*(1), 1-2.
3. Revathi, m., Karthikeyan, m., Gnanasekaran, a., &ms, r. (2018). screening of antimicrobial activity of siddha drug kukkiladhichoornam.*hygeia.j.d.med.*10 (1); 40-42.
4. Saravanasingh, K., David, E., Parthiban, P., & Ramamurthy, M. (2021). In vitro and in Vivo Antimicrobial Activity of Padigaraparpam Against Staphylococcus Petrasii Subsp. Pragensis, Recently Identified Pathogen. *Annals of the Romanian Society for Cell Biology*, 17300-17306.
5. Shobha K.L, Akshatha S.J, Revathi Shenoy, Ramachandra L &Bernitis L.,(2014) "Antibacterial Activity of Selected Siddha herbo-mineral Preparations Against Clinically Isolated Enterococcus Species" *International Journal of Pharmacology and Toxicology* 2.2 : 38-40.
6. Suvetha C1*, Seetha Lakshmi G2 , Vanitha, A3, (2018). "Antimicrobial screening of a Siddha formulation BrahmananthaBairavamMaathirai*" Int. J. Adv. Res. Biol. Sci.* 5(1): 22-24
7. Purushotham, K. G., Arun, P., Jayarani, J. J., Vasnthakumari, R., Sankar, L., & Reddy, B. R. (2010). Synergistic in vitro antibacterial activity of Tectona grandis leaves with tetracycline. International journal of pharmtech research, 2(1), 519-523.

8. Michael, J. S., Singh, A. R., &Padmalatha, C. (2011). Antibacterial potential of some herbo-mineral siddha preparation: An alternative medicine for enteric pathogens. J. Chem. Pharm. Res, 3(3), 572-578.
9. Rajalakshmi, K. (2016). Screening of common siddha formulations for antimicrobial activity against respiratory pathogens. Screening, 9(2).
10. Akilan, A., & Revathi, K. (2020). Antibacterial Activity Of Selected Siddha Preparations Against Clinically Important Standard Reference Bacterial Strains. Annals of the Romanian Society for Cell Biology, 1145-1151.
11. Uma K.S., Jacob M., Arumugam G., Kalpana S. and Natarajan M (2012) In Vitro Antimicrobial Activity of the Siddha Drugs Seenthil Sarkarai and Nilavembu Kudineer against Leptospira. International Journal of Pharmacy and Pharmaceutical Sciences. Vol 4, Suppl 2.
12. Handral H.K., Pandith A., Shruthi S. D (2012). A review on murraya koenigii: multipotential medicinal plant. Asian Journal of Pharmaceutical and Clinical Research .Vol 5, Suppl 4.

Tables

Table 1. A display of the anti-bacterial effect of Padikara Parpam against *E. coli* and *K. pneumonia* at various concentrations

ZONE OF INHIBITION	0.50%	1.00%	1.50%	2.00%	CTX 30 (CONTROL)	AMPC 30 (CONTROL)
Escherichia Coli	14 mm	17mm	19mm	22mm	16mm	16mm
Klebsiella Pneumonia	10mm	14mm	17mm	20mm	20mm	21mm

Figures

Figure 1: Isolation and identification of *E. Coli* and *K. Pneumonia*

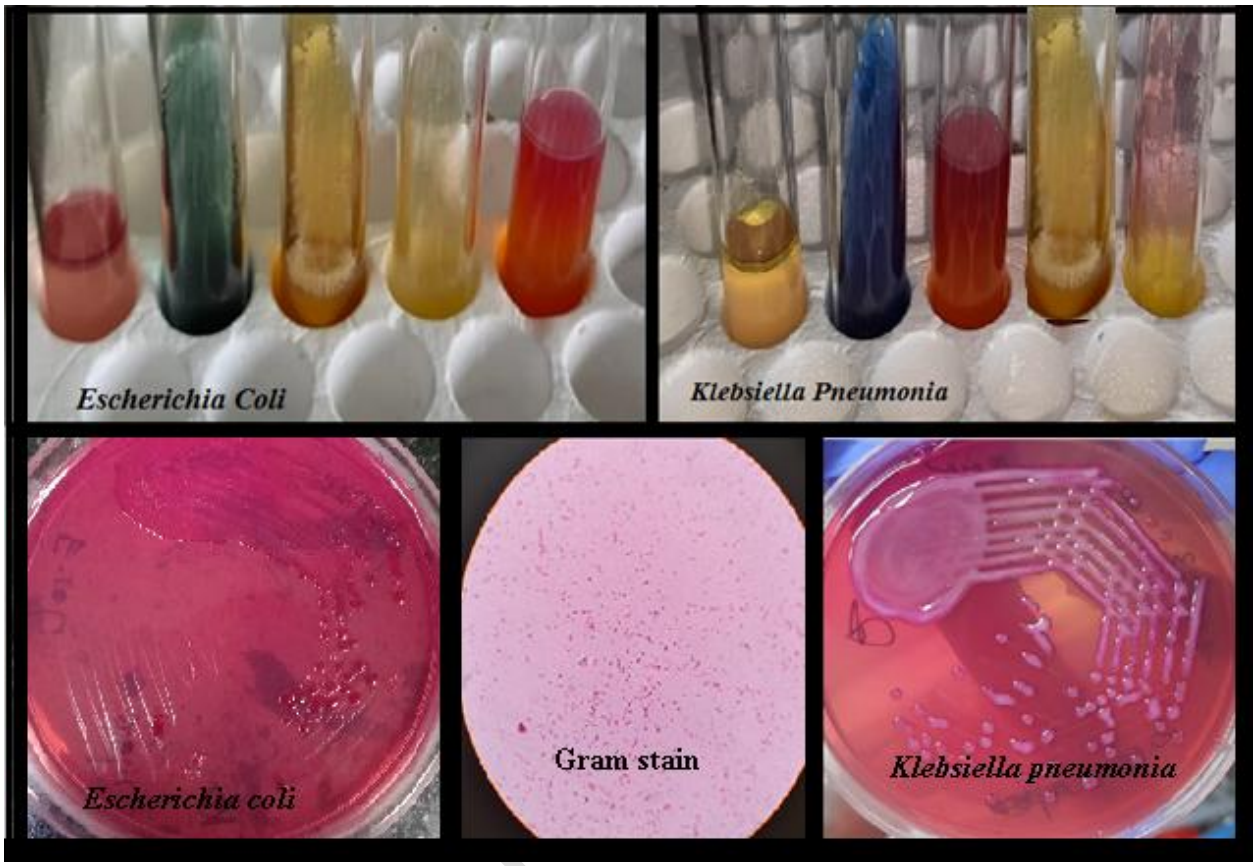


Figure 2: Antibacterial activity of Padikara Parpam against *E. coli* and *K. pneumonia*

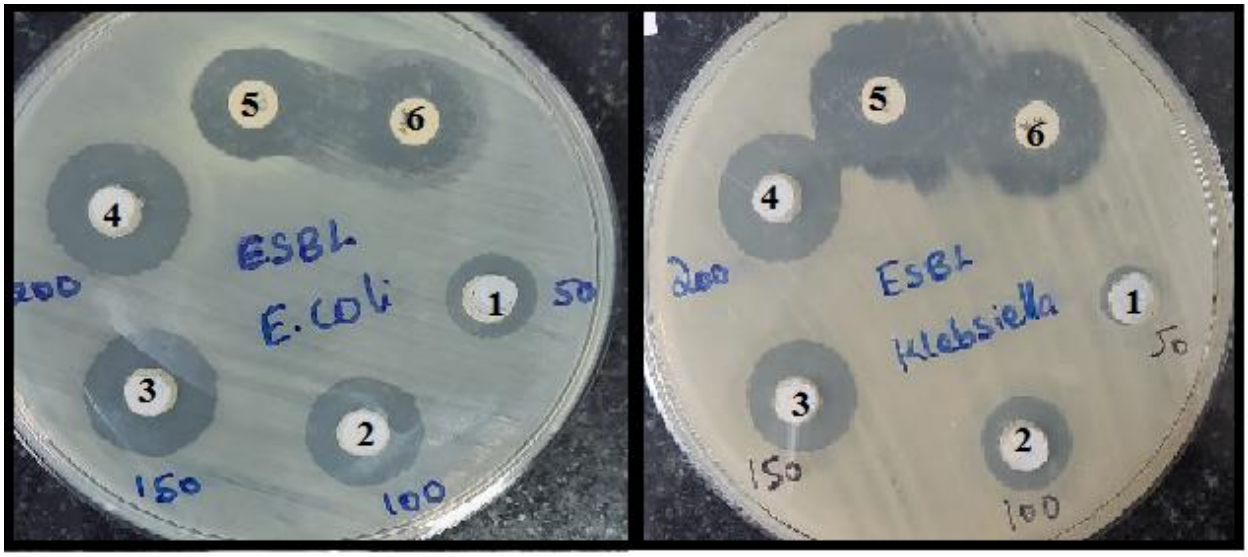
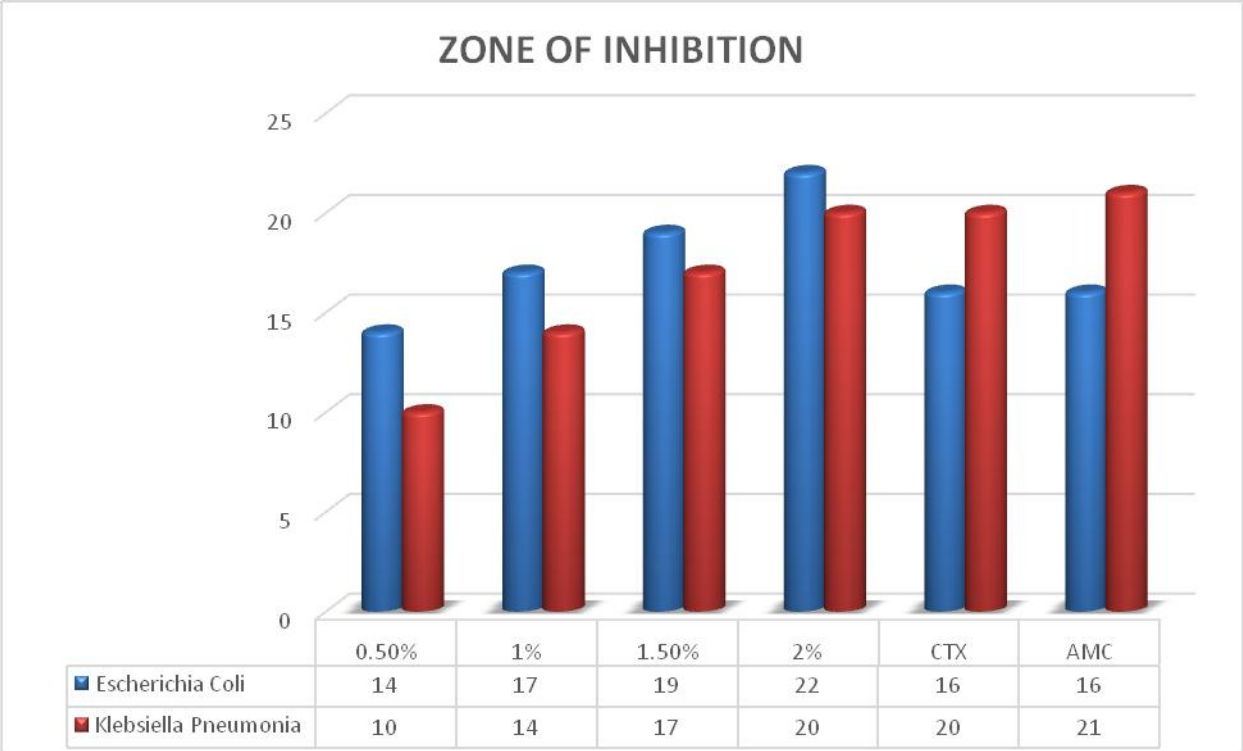


Figure 3. Graphical representation showing the potential of Padikara Parpam as anti-bacterial agent in ESBL producing bacteria



	0.50%	1%	1.50%	2%	CTX	AMC
Escherichia Coli	14	17	19	22	16	16
Klebsiella Pneumonia	10	14	17	20	20	21