

ANTIBACTERIAL ACTIVITY OF PIPER LONGUM AGAINST MULTIPLE DRUG RESISTANT KLEBSIELLA PNEUMONIAE**Sadhana Jaiswal**Department of Microbiology, Government Nagarjuna P.G. College of Science, Raipur, C.G., India
skaushaljaiswal123@gmail.com**ABSTRACT**

Antibiotic resistance in pathogens and commensal organisms is likely a result of the overuse of antibiotics in a range of industries, including pharmaceutical, medical, and animal husbandry. It poses a significant threat to contemporary clinical procedures since it may result in unfavourable treatment outcomes, monetary losses, and the emergence of a human-transmissible gene pool. One possibility that may have an effect on microbial resistance is the usage of medicinal herbs. In Indian culture, spices and herbs have been utilized as food additions, flavouring or colouring agents, and preservatives. *Piper longum* (long pepper) is well known Indian spice has medicinal and pharmaceutical importance as it is reported to have a variety of bioactive compounds such as piperine, piperlongumine, sylvatin, sesamin, diaeudesmin piperlonguminine, pipermonaline, and piperundecalidine. It is used in various medicinal systems like Ayurveda and Shiddha, Unani etc. to cure many disorders. The current study is aimed to access the efficacy of extracts of *Piper longum* in different solvents against multidrug-resistant *Klebsiella pneumoniae* isolated from pond water.

Keywords: MDR, *Piper longum*, *Klebsiella pneumoniae*, long pepper, antimicrobial

INTRODUCTION

The emergence of new strains of disease-causing organisms and the development and spread of antibiotic resistance are major concerns for the global health community. Many spices have been used as herbal remedies since ancient times due to their medicinal properties. In order to treat illnesses brought on by bacteria and other pathogens, these medicinal plants are an invaluable resource for naturally occurring antimicrobial substances. In developing countries about 80% of the population depends on traditional medicines for their health related problems (Kim, 2005). One such plant is *Piper longum*, commonly known as Thippali, is a native to the Indo-Malaya region, belongs to the Piperaceae family. Its name is derived from the Tamil word for long pepper, "pippali." Traditionally used in Indian cuisine, the dried fruits of *P. longum* serve as a spice and seasoning, and it holds significant importance in Ayurvedic medicine. Its fruits contain various phytoconstituents, including volatile oils, starch, proteins, alkaloids, saponins, and carbohydrates (Dasgupta & Dutta, 1980). Key active compounds identified in *P. longum* include piperine, piperlongumine, sylvatin, sesamin, diaeudesmin, piperlonguminine, pipermonaline, and piperundecalidine (Dutta et al., 1975, Gani et al., 2019). This plant is renowned for its benefits to the respiratory system, metabolism, and digestion, it is also reported as a good remedy to cure menstrual pain, gonorrhoea, sleeping problems, tuberculosis, respiratory tract infections, arthritic conditions and chronic gut-related pain. The roots and fruits of *P. longum* are used to treat fever, asthma, hemorrhoid infection, bronchial stress, abdominal pain, inflammation, jaundice diarrhea and antidote to snake bite (Mehta et al., 1998).

The primary active ingredient in *Piper longum* plants piperine, has been shown to have hepatoprotective, analgesic, antioxidant, antipyretic, CNS depressant, and anti-inflammatory effects (Wakade et al., 2008; Ratner et al., 1991; Gurumurthy et al., 2012). In Ayurveda, it is known for quite effective in treating dipaniya (stomachic) and pachaniya (digestive). According to a number of clinical research, pippali significantly reduces children's bronchial asthma (Dahanukar et al., 1984). Das et al., 2022; Dinesha & Chikkanna 2014, reported the antioxidant activities of *Piper longum*. Present study have highlighted the antibacterial properties of *P. longum*. specifically, against multi-drug resistant *Klebsiella pneumoniae*, isolated from pond water, in various solvent extracts—namely, water, methanolic, and ethanolic. This research underscores the potential of *Piper longum* as a natural antimicrobial agent.

MATERIAL AND METHOD

A total of 10 bacterial strains were isolated from water samples of various ponds of Raipur city by following the standard methods using EMB agar medium. Pure cultures of isolates were preserved at 4°C on nutrient agar slants. Antibiotic Susceptibility test was performed in Mueller Hinton agar media, using the disk diffusion method against 12 common antibiotics namely Ampicillin (AMP), Cefazolin (CZ) nalidixic acid (NA), Streptomycin (S), Sulphafurazole (SF), Tetracycline(TE), Kanamycin (K), Gentamycin (GEN), Chloramphenicol(C), Erythromycin(E), Neomycin (N) and Amoxycylav (AMC). Out of the 10 bacterial isolates, the one who shows resistance against maximum number of antibiotics was chosen for further study. *Effect of Piper longum on this MDR strain was assessed by following the well diffusion method.* A well filled with the respective solvent served as a negative control. *The extracts of the Piper longum was prepared in three solvents- water, methanol & ethanol. The cleaned and dried fruits of Piper longum was finely ground, 2 grams of powder was added to 20ml distilled water /methanol/ethanol and kept as such for 48 hours in dark with intermittent shaking. After 48 hours the mixture was centrifuged at 10000rpm for 10minutes, the supernatant was concentrated in water-bath and utilized for susceptibility studies.* The antibacterial activities of the extracts were determined by measuring the diameter of the zone of inhibition in mm. All the experiments were performed in triplicate (three independent experiments).

RESULT AND DISCUSSION**Table 1:** Antibiogram of the bacterial strains isolated from Pond water.

Antibiotics	Bacterial isolates									
	1	2	3	4	5	6	7	8	9	10
Ampicillin	S	R	R	S	S	R	R	R	S	R
Cefazolin	S	S	S	S	S	S	S	S	S	S
Nalidixic	S	S	S	S	S	S	S	S	S	S
Streptomycin	S	S	R	S	S	S	R	S	S	S
Sulphafurazole	S	S	R	S	S	S	S	S	S	S
Tetracycline	S	R	S	S	S	S	S	S	S	S
Kanamycin	S	S	S	S	S	S	S	S	S	S
Gentamicin	S	S	S	S	S	S	S	S	S	S
Chloramphenicol	S	R	S	S	S	S	S	S	S	S
Erythromycin	S	S	R	S	S	R	S	S	S	S
Neomycin	S	S	S	S	S	S	S	S	S	S
Amoxiclav	R	R	R	R	R	S	R	R	S	R

R(resistant), S (sensitive)

Antibiogram study of the ten isolated strains is depicted in table 1. Isolate no. 3 showed resistance against maximum (5 out of 12) antibiotics used and gives the highest MAR index 0.42. Due to its multidrug resistance, this isolate was selected for the study of antimicrobial effect of *Piper longum* extracts.

Molecular Characterization of MDR Strain

The isolate 3 showing resistance against maximum antibiotics was subjected to molecular characterization and identified using 16 S rRNA as *Klebsiella pneumoniae* strain DSM 30104 by Biokart India Pvt. Ltd., Bengaluru, Karnataka.

Aligned Sequence Data of Sample–HP3(1287bp)

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P4GATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACGATCGGTTT
TATGGGATTAGCTCCACCTCGCGGCTTGGCAACCCTTTGTACCGACCATTGTAGCACGTGTGTAGCC
CTGGCCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCCTCCGGTTTGTACCCGGCAGTCTC
CTTAGAGTGCCACCCGAGGTGCTGGTAACTAAGGACAAGGGTTGCGCTCGTTACGGGACTTAACC
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CAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTCTGAGTTCCCGAAGGCACCA
 ATCCATCTCTGAAAGTTCTCAGCATGTCAAGGCCAGGTAAGGTTCTTCGCGTTGCTTTCGAATTA
 CCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCCGTACTC
 CCCAGGCGGTTCGACTTATCGCGTTAGCTGCGCCACTAAGATCTCAAGGATCCCAACGGCTAGTCGAC
 ATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGTCCCCACGCTTTCGCACCTCAGTGT
 CAGTATCAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCTATATCTACGCATTTACCCGCTAC
 ACAGGAAATTCCACCACCCTCTACCGTACTCTAGCTCAGTAGTTTTGGATGCAGTTCCAGGTTGAG
 CCCGGGGATTTACATCCAACCTTGCTGAACCACCTACGCGCGCTTACGCCAGTAATTCCGATTAA
 CGTTGCACCCTTCGTATTACCGCGGGTGTGGCACGAAGTTAGCCGGTGTATTCTGTTGGTAAC
 GTCAAAACAGCAAGGGAATTAACCTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAG
 ACCTTCTTACACACGCGGCATGGCTGGATCAGGCTTTCGCCATTGTCCAATATTCCCACTGCTGC
 CTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCAGTGTGACTGATCATCCTCTCAGACCAGTTACGG
 ATCGTCGCCTTGGTAGGCCTTACCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGTG
 AGGTCCGAAGATCCCCACTTCTCCCTCAGGACGTATGCGGTATTAGCGCCCGTTTCCGGACGTTA
 TCCCCACTACCAGGCAGATTCTAGGCATTACTACCCGTCGG

DNA Sequencing of Isolate No. 3

Phygenetic Tree

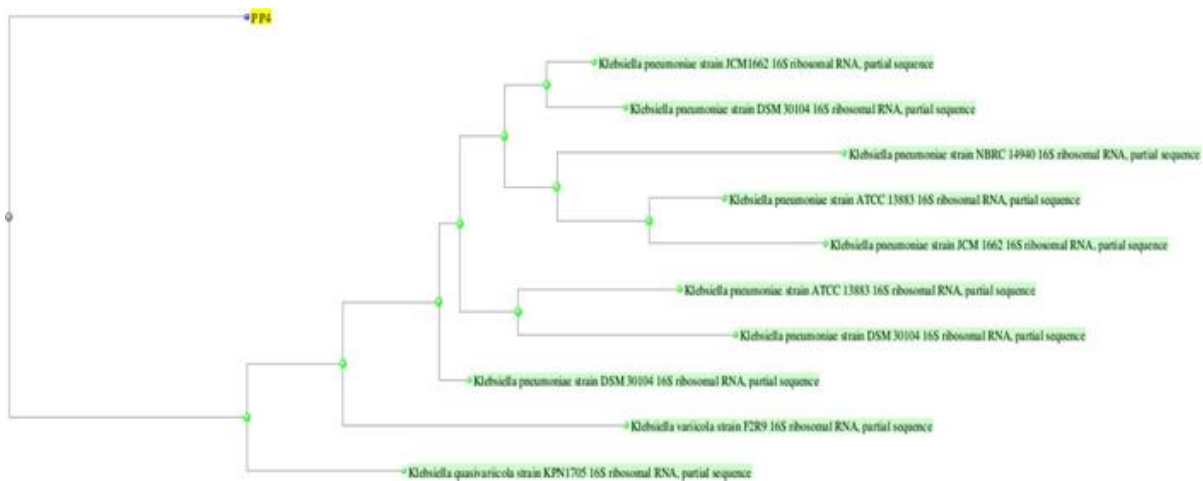


Table 2: Blastdata

Sl. No.	OrganismName	Accession No.	%Match
1	Klebsiella pneumoniae strain DSM30104 16S ribosomal RNA	NR_117683.1	99.49%
2	Klebsiella pneumoniae strain ATCC13883 16S ribosomal RNA	NR_114506.1	99.41%
3	Klebsiella quasivariicola strain KPN1705 16S ribosomal RNA	NR_181901.1	99.34%
4	Klebsiella pneumoniae strain DSM30104 16S ribosomal RNA	NR_036794.1	99.34%
5	Klebsiella pneumoniae strain ATCC13883 16S ribosomal RNA	NR_119278.1	99.34%
6	Klebsiella pneumoniae strain JCM 1662 16S ribosomal RNA	NR_112009.1	99.34%
7	Klebsiella pneumoniae strain DSM30104 16S ribosomal RNA	NR_117686.1	99.34%
8	Klebsiella variicola strain F2R9 16S ribosomal RNA	NR_025635.1	99.34%
9	Klebsiella pneumoniae strain JCM1662 16S ribosomal RNA	NR_113240.1	99.27%
10	Klebsiella pneumoniae strain NBRC 14940 16S ribosomal RNA	NR_113702.1	99.27%

Antimicrobial Activity of Spices against MDR *Klebsiella pneumoniae*

Antibacterial efficacy of *Piper longum* against multi drug resistant *Klebsiella pneumoniae* was tested in three different kinds of extracts which were made independently with water, methanol, and ethanol. While most conventional herbal therapies use water, which is a readily available and ubiquitous solvent, this study also included two organic solvents, methanol and ethanol, for more effective extraction of the majority of the bioactive compounds that may have antimicrobial properties. The results of the agar well diffusion test for antibacterial screening showed that the extracts of *Piper longum* with different solvents displayed varying degrees of growth inhibition, The sequence of antibacterial activity of *Piper longum* extracts against MDR *Klebsiella pneumoniae*, based on the observed zones of inhibition was as follows:

Methanol (25mm) > Ethanol (14mm) > Aqueous extract (5mm)

The information provided validates the theory that *Piper longum* has an inhibiting impact on MDR bacteria. The result shows that the methanolic extract of *Piper longum* exhibited significant antibacterial activity against *Klebsiella pneumoniae*, while the crude aqueous extracts displayed the least amount of antibacterial activity. This pattern suggests that the methanolic extract is the most potent in exhibiting antibacterial properties against MDR *Klebsiella pneumoniae*, reinforcing the potential of *Piper longum* as a source of antibacterial compounds. Further studies could explore the specific compounds responsible for this activity and their mechanisms of action. These results are perfect correlation with the study of Dash et al. in 2022, who found that volatiles isolated from dried *Piper longum* fruit and leaves had a potent antimicrobial effect against *Klebsiella pneumoniae* and *Acinetobacter baumannii*. Similar findings by Sultana, et al. 2019 which reports the antibacterial properties of *Piper longum* against *Staphylococcus aureus* and *Salmonella typhi*. Rawat et al., 2022 investigated the antibacterial, antioxidant, and phytochemical analysis of *Piper longum* fruit extracts against multi-drug resistant non-typhoidal *Salmonella* strains. Sindhu et al., 2019 found the antibacterial activity of *Piper longum* against both gram positive and gram negative bacteria. Various studies of antimicrobial activities of different spices were investigated by many researchers, Vyas et al. (2015) reported the antifungal and antibacterial properties of cinnamon's methanolic extract against *Candida albicans* and *S. aureus* and *E. coli*. In a different study conducted by Vaz et al. in 2022, secondary plant metabolites from *Zingiber officinale*'s showed strong antibacterial properties against 18 resistant pathogens with high efficacy against gram-negative MDR pathogens, like carbapenem- and polymyxin-resistant *Klebsiella pneumoniae* and *Serratia marcescens*. Another study by Mickymaray, & Al Aboody (2019) reported the Spice-extracted essential oils have been showed their antibacterial activity against pathogenic strains isolated from UTI patients. This demonstrates that using spices as a substitute can be a successful way to fight infections linked to MDR. By employing the active components of the spices, future research can be conducted to clarify the molecular mechanisms underlying the antibacterial action & antibiotic resistance reversal.

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