The Bioscan 19(2) S2: 97-100, 2024 www.thebioscan.com
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

ANTIBACTERIAL ACTIVITY OF SPICES AGAINST MULTIPLE DRUG RESISTANT PSEUDOMONAS AERUGINOSA ISOLATED FROM POULTRY WASTE WATER

SADHANA JAISWAL[1], *DEEPALI RAJWADE[2], DIKSHA KHARE[3], SHRIJAL KUMAR SHARMA[4], ALKA KAUSHIK[5]

Government N.P.G.College of Science Raipur, Chhattisgarh-492001

Corresponding author

Email: ddrajwade@gmail.com

DOI: https://doi.org/10.63001/tbs.2024.v19.i02.S2.pp97-100

KEYWORDS
MDR,
Pseudomonas aeurigenosa,
poultry waste water,
cinnamon,
black pepper,
long pepper.

Received on:

08-04-2024

Accepted on:

05-09-2024

Corresponding author

ABSTRACT

Conventionally spices have been widely used as flavoring agents, colorant, preservatives, food additives and medicinal purposes worldwide. The present work aimed to find out the antimicrobial activity of natural spices on multi-drug resistant *Pseudomonas aeurigenosa* isolated from poultry waste water. Anti-bacterial potentials of four spices extracts cinnamon, clove, black pepper and long pepper) were tested against *Pseudomonas aeurigenosa* isolated from poultry waste water.The highest inhibition zone was observed with long pepper (32mm). Assessment of anti-bacterial activity of common spices against other MDR bacteria could be applied for prevention & mitigation of diseases.

INTRODUCTION

Antimicrobial resistance or multidrug resistance is a condition in which microbes develop resistance against a variety of conventional antibiotics. It has quickly emerged, persisted, and spread as a consequence of improper use of antibiotics in healthcare. The medical community now has limited therapeutic alternatives available for managing these strains. Antibiotic-resistant pathogen infections typically result in significant morbidity and mortality as well as a significant financial burden on the world's healthcare system (Centres for Disease Control and Prevenation, 2019). Regretfully, in community-acquired illnesses, MDR microorganisms are increasingly being identified Antimicrobial resistance (AMR) is a natural phenomenon, driven by several factors that contribute to its emergence and spread (Depta et al., 2023). These include the indiscriminate use of antimicrobial drugs, substandard drug quality (Clifford et al., 2018), and the inappropriate use of these drugs in agriculture and livestock as feed additives and growth promoters (Silveira et al., 2021). The subsequent contamination of soil, sediments, and water bodies further accelerates the development and spread of antibiotic- resistant strains in the environment (Gothwal and Shashidhar, 2015; Said *et al.*, 2016). Moreover, waste from pharmaceutical industries, hospitals, and livestock production contains unmonitored quantities of antimicrobials, which promote multidrug resistance in the environment, potentially leading to the passing of resistance genes to human beings and other animals (Oyekale and Oyekale, 2017; World Bank, 2017). Given the rapid emergence of drug resistance and its severe threat to global health, developing new drugs to combat these infections is a top priority for the World Health Organization (WHO, 2021, The Lancet Infectious Diseases, 2017). WHO has published its first-ever list of antibiotic-resistant priority pathogens, highlighting twelve bacterial families that pose the greatest threat to human health (World Health Organization, 2017). This list categorizes resistant pathogens into three tiers based on the urgency of developing new antibiotics. The first group, critical priority pathogens, includes *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and members of the Enterobacteriaceae family (*Klebsiella*, *Escherichia coli*, *Serratia*, and *Proteus*). The second group, classified as high priority, includes pathogens such as *Enterococcus faecium*, *Staphylococcus aureus*, *Helicobacter pylori*, *Campylobacter* spp., *Salmonellae*, and *Neisseria gonorrhoeae*. The final group, medium priority, consists of pathogens like *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Shigella* spp.It is now widely recognized that long-term reliance on antibiotics is unsustainable, making the pursuit of alternative strategies to counter microbial infections essential.

Phytochemicals for combating antibiotic resistance

Higher plants naturally synthesize a diverse array of secondary metabolites with significant therapeutic potential, including polyphenols, terpenoids, sulfides, coumarins, saponins, furans, alkaloids, polyynes, and thiophenes (Mehreen et al., 2016). Many of these phytochemicals exhibit antimicrobial activity, as they are produced by plants as a defense mechanism against microbial invasion. Numerous phytochemicals have been validated for their antimicrobial potential, including effectiveness against multidrug-resistant (MDR) strains (Shriram et al., 2018; Anand et al., 2019; Yu et al., 2020). Some of the Indian spices extends beyond their culinary applications to their traditional use as therapeutics. Recent studies have provided scientific evidence supporting the bioactivities of various spices. Extensive research has also identified the active phytochemicals within these spices, explored their antimicrobial antiparasitic, anthelmintic, analgesic, expectorant, sedative, antiseptic, and antidiabetic properties, and investigated their potential as therapeutic drugs. (Adamczak et al., 2020; Choo et al., 2020; Rahmani et al., 2017, Buathong, 2023). The aim of present study is to investigate the antibacterial effect of four Indian spices namely Clove, Cinnamon, Long pepper and Black pepper against multidrug-resistant *Pseudomonas aeurigenosa* isolated from poultry waste water.

Materials and methods

A total of 10 bacterial strains were isolated from poultry waste water of various region of Raipur city by standard methods in Eosin Methylene Blue Agar media. Pure cultures of isolates were preserved at 4˚C on nutrient agar slants. Morphological &biochemical tests were carried out to identify the test bacterial isolates. Antibiotic Susceptibility test was performed in Mueller Hinton agar media, using the disk diffusion method against 12 common antibiotics namely Ampicillin 25mcg (AMP), Cefazolin 30mcg (CZ), Nalidixic Acid 30mcg (NA), Streptomycin 25mcg (S), Sulphafurazole 300mcg (SF),Tetracycline 30mcg (TE),Kanamycin 30mcg (K), Gentamicin 10mcg (GEN), Chloramphenicol 10mcg (C), Erythromycin 10mcg (E), Neomycin 30mcg (N), Amoxyclav 20mcg (AMC). Among the 10 isolates, further study was carried out with the isolate showing resistance against maximum number of antibiotics. Effect of various spices on this MDR strain was assessed using well diffusion method following standard method. A well filled with the respective solvent served as a negative control. Total four spices were selected for this study- Clove, Cinnamon, Long pepper & black pepper. The extracts of the spices was prepared in three solvents- water, methanol & ethanol. The cleaned and dried spices were finely ground. For preparation of extract, 2 grams each of the spices 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 10 minutes and the supernatant was utilized for antibacterial effect. 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). Statistical analysis of the observation is done by two way ANOVA to compare the various solvents and spices effect.

RESULTS AND DISCUSSION

Various biochemical tests were performed for preliminary identification of the bacterial strains isolated from the poultry waste water (Table 1).

Table.1Biochemical Characterization of bacterial isolates

Isolates	IMVIC TEST				TSIA TEST						Urease	Catalase	Oxidase
	Indole Test	MR test	VP Test	Citrate test	Colour of Slant/Butt	Lactose fermentation	Fructose Fermentation	Dextrose Fermentation	Gas Production	H2S production			
1.	-	-	-	+	Y/B	+	+	-	+	+	-	+	+
2.	-	+	-	+	Y/B	-	-	+	+	+	-	+	-
3.	-	+	-	+	Y/Y	-	-	+	+	-	-	+	-
4.	-	-	+	+	Y/Y	+	+	+	-	-	-	+	-
5.	+	+	-	-	R/B	-	-	+	-	+	-	+	-
6.	-	-	-	+	R/Y	-	-	-	+	-	-	+	+
7.	-	-	-	+	R/Y	+	+	+	+	-	-	+	+
8.	-	-	-	+	Y/Y	-	-	+	-	-	+	+	+
9.	+	-	+	+	Y/B	+	+	+	-	+	-	+	+
10.	-	-	-	+	R/Y	-	-	-	+	-	-	+	+

Y stand for yellow, R stand for red and B stand for black

Table No. 2 Conventional bacterial identification by ABIS online

Isolates	Probable organism	Score	Isolates	Probable organism	Score
1	*Camphylobacter jejuni*	100%	6	*Pseudomonas aeruginosa*	100%
2	*Salmonella enteritidis*	95%	7	*Camphylobacter sp.*	100%
3	*Salmonella sp.*	95%	8	*Brucella sp.*	90%
4	*Enterobacter sp.*	98%	9	*Aeromonas sp.*	86%
5	*Escherichia coli*	72%	10	*Pseudomonas sp.*	100%

Table 3. Antibiogram of the isolated strains

Isolates / Antibiotic	ZONE OF INHIBITION (mm)									
	1	2	3	4	5	6	7	8	9	10
Ampicillin	Nil	10mm	Nil	Nil	nil	14mm	15mm	nil	15mm	15mm

Cefazolin	13mm	15mm	15mm	20mm	18cm	Nil	20mm	15mm	20mm	25mm
Nalidixic	20mm	20mm	13mm	15mm	17cm	15mm	15mm	20mm	13mm	20mm
Streptomycin	14mm	20cm	20mm	20mm	20cm	20mm	2mcm	20mm	20mm	20mm
Sulphafurazole	Nil	15mm	25mm	20mm	25cm	Nil	25mm	10mm	20mm	20mm
Tetracycline	23mm	20mm	20zm	18mm	15cm	20mm	20mm	15mm	23mm	20mm
Kanamycin	20mm	10mm	15cm	17mm	15cm	13mm	15mm	10mm	20mm	15mm
Gentamicin	20mm	20mm	10cm	20mm	18cm	20mm	25mm	20mm	20mm	25mm
Chloramphenicol	10mm	10mm	Nil	20mm	20cm	Nil	20mm	10mm	20mm	20mm
Erythromycin	25mm	14mm	10cm	15mm	10cm	Nil	14mm	10mm	18mm	25mm
Neomycin	22mm	15mm	15mm	20mm	20cm	20mm	15mm	2mm	15mm	20mm
Amoxyclav	10mm	Nil	12mm	nil	18cm	Nil	18mm	15mm	20mm	20mm

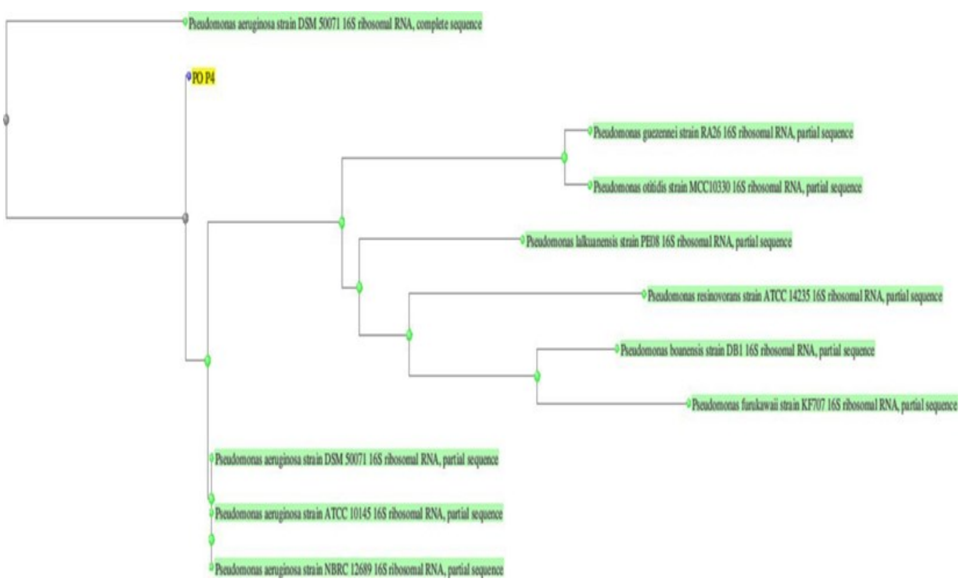
On the basis of morphological and biochemical characteristics the probable identification of bacterial isolates were done using an online tool ABIS (Table 2).

Antibiogram study of the ten isolated strains is depicted in table 3. The isolate 6 showed resistance against maximum (5 out of 12) antibiotics used. Due to its multidrug resistance, this isolate was subjected to molecular characterization and selected for the study of antimicrobial effect of the selected spices extract and was identified using 16 S rRNA as *Pseudomonas aeruginosa* by Biokart India Pvt. Ltd., Bengaluru, Karnataka.

. Aligned Sequence Data of Sample–(1250bp)

>POP4GATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACT
GCGATCCGGACTACGATCGGTTTTATGGGATTAGCTCCACCTCGCG
GCTTGGCAACCCTTTGTACCGACCATTGTAGCACGTGTGTAGCCCT
GGCCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCG
GTTTGTCACCGGCAGTCTCCTTAGAGTGCCCACCCGAGGTGCTGGT
AACTAAGGACAAGGGTTGCGCTCGTTACGGGACTTAACCCAACATC
TCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTCTGAGTT
CCCGAAGGCACCAATCCATCTCTGGAAAGTTCTCAGCATGTCAAGGC
CAGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACC
GCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGC
CCGTACTCCCCAGGCGGTCGACTTATCGCGTTAGCTGCGCCACTAA
GATCTCAAGGATCCCAACGGCTAGTCGACATCGTTTACGGCGTGGA
CTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCA
GTGTCAGTATCAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTT
CCTATATCTACGCATTTCACCGCTACACAGGAAATTCCACCACCCTC
TACCGTACTCTAGCTCAGTAGTTTTGGATGCAGTTCCCAGGTTGAG
CCCGGGGATTTCACATCCAACTTGCTGAACCACCTACGCGCGCTTTA
CGCCCAGTAATTCCGATTAACGCTTGCACCCTTCGTATTACCGCGGC
TGCTGGCACGAAGTTAGCCGGTGCTTATTCTGTTGGTAACGTCAAA
ACAGCAAGGGAATTAAACTTACTGCCCTTCCTCCCAACTTAAAGTGC
TTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAG
GCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAG
TCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACC
AGTTACGGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTA
ATCCGACCTAGGCTCATCTGATAGCGTGAGGTCCGAAGATCCCCCA
CTTTCTCCCTCAGGACGTATGCGGTATTAGCGCCCGTTTCCGGACG
TTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCC
G

- The Microbe was found tobe Pseudomonas aeruginosastrain DSM50071 16SribosomalRNA
- SequenceID:NR_117678.1
- The next closest homologue was found tobe Pseudomonas aeruginosa strain NBRC12689 16S ribosomal RNA



- SequenceID:NR_113599.1

Spice extracts were separately prepared using ethanol, Methanol and water. Although Water is a common and easily available solvent for most of the conventional herbal treatment, but it cannot efficiently extract the most of the secondary metabolites which are antibacterial in nature. Due to this polar solvents like methanol & ethanol were included in this study. Antibacterial screening revealed that crude extracts of all the four spices displayed antibacterial activity against *P. Aeruginosa* in one or the other extract. Methanol extract of long pepper showed maximum inhibitory activity (Table 4). Statiscal analysis showed there is no any significant difference between the methanol and ethanol extracts.

Table 4 Antimicrobial activity of spices against MDR *P .aeruginosa*

Spices	Zone of Inhibition (mm)	
	Methanol Extract	Ethanol Extract
Clove Cinnamon Long pepper Black pepper	15mm 21mm 32mm 10mm	17mm 10 mm 15mm 13mm

The data given in the above table supports the assumption that some common Indian spices have an inhibitory effect on even multiple drug resistant bacteria. The results suggest that clove, cinnamon and long pepper have significant antimicrobial activity. Vyas et al. (2015) reported antibacterial activity of methanolic extract of cinnamon against *S. aureus* and *E. coli,* as well as antifungal activity against *Candida albicans*, which is similar to the findings of this investigation. Similar results were also reported by Dash et al., (2022) wherein extracted volatiles from dried leaf and fruit of *Piper longum* showed a very strong activity against MDR *Acinetobacter baumannii* & *Klebsiella pneumoniae*. In another study by Vaz et al., (2022), secondary plant metabolites like 1,8-cineole, camphene, β-phellandrene, and α-curcumene obtained from the essential oil of Zingiber officinale demonstrated significant 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 Mehta el al.,(2022) reported the antibacterial efficacy of secondary metabolites (lariciresinol and berberine) of *Zingiber offcinale* Roscoe of the North-Western Himalaya region against MDR *Salmonella typhimurium, P.longum* extracts also have the ability to increase the effectiveness of different classes of antibiotics and reverse their resistance against multidrug-resistant *Staphylococcus aureus* (Din et al., 2023).This shows that spices can be an efficient alternative to combat MDR associated infections. Future studies can be undertaken to elucidate the molecular mechanisms behind the antibacterial activity & antibiotic resistance reversal utilizing the active ingredients of the spices.

REFERENCES

- Anand, U., Jacobo-Herrera, N., Altemimi, A. and Lakhssassi, N., 2019. A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery. *Metabolites*, *9*(11), p.258.
- Adamczak, A., Ożarowski, M. and Karpiński, T.M., 2020. Curcumin, a natural antimicrobial agent with strain-specific activity. *Pharmaceuticals*, *13*(7), p.153.
- Buathong, R. and Duangsrisai, S., 2023. Plant ingredients in Thai food: a well-rounded diet for natural bioactive associated with medicinal properties. *PeerJ*, *11*, p.e14568.
- Centers for Disease Control and Prevenation, 2019. Biggest Threats and Data Antibiotic/Antimicrobial Resistance| CDC. Available at: https://www.cdc.gov/ Drug resistance/biggest-threats.html (Accessed February 12, 2021).
- Choo, S., Chin, V.K., Wong, E.H., Madhavan, P., Tay, S.T., Yong, P.V.C. and Chong, P.P., 2020. Antimicrobial properties of allicin used alone or in combination with other medications. *Folia Microbiologica*, *65*, pp.451-465.
- Clifford, K., Desai, D., da Costa, C.P., Meyer, H., Klohe, K., Winkler, A.S., Rahman, T., Islam, T. and Zaman, M.H., 2018. Antimicrobial resistance in livestock and poor quality veterinary medicines. *Bulletin of the World Health Organization*, *96*(9), p.662.
- Dash, M., Singh, S., Sahoo, B.C., Sahoo, S., Sahoo, R.K., Nayak, S. and Kar, B., 2022. Potential role of Indian long pepper (Piper longum L.) volatiles against free radicals and multidrug resistant isolates. *Natural product research*, *36*(16), pp.4271-4275.
- Depta, J. and Niedźwiedzka-Rystwej, P., 2023. The phenomenon of antibiotic resistance in the polar regions: an overview of the global problem. *Infection and drug resistance*, pp.1979-1995.
- Din, M.S.U., Gohar, U.F., Mukhtar, H., Khan, I., Morris, J., Pornpukdeewattana, S. and Massa, S., 2023. Antibiotic Reversal Activity of Piper longum Fruit Extracts against Staphylococcus aureus Multi-Drug Resistant Phenotype. *Microbiology and Biotechnology Letters*, *51*(4), pp.432-440.
- Gothwal, R. and Shashidhar, T., 2015. Antibiotic pollution in the environment: a review. *Clean–Soil, Air, Water*, *43*(4), pp.479-489.
- Mehreen, A., Waheed, M., Liaqat, I. and Arshad, N., 2016. Phytochemical, antimicrobial, and toxicological evaluation of traditional herbs used to treat sore throat. *BioMed research international*, *2016*(1), p.8503426.
- Mehta, J., Rolta, R. and Dev, K., 2022. Role of medicinal plants from North Western Himalayas as an efflux pump inhibitor against MDR AcrAB-TolC Salmonella enterica serovar typhimurium: In vitro and In silico studies. *Journal of Ethnopharmacology*, *282*, p.114589.
- Rahmani, A.H., Alsahli, M.A. and Almatroodi, S.A., 2017. Active constituents of pomegranates (Punica granatum) as potential candidates in the management of health through modulation of biological activities. *Pharmacognosy Journal*, *9*(5).
- Oyekale, A.S. and Oyekale, T.O., 2017. Healthcare waste management practices and safety indicators in Nigeria. *BMC public health*, *17*, pp.1-13.
- Said, L.B., Jouini, A., Alonso, C.A., Klibi, N., Dziri, R., Boudabous, A., Slama, K.B. and Torres, C., 2016. Characteristics of extended-spectrum β-lactamase (ESBL)- and pAmpC beta-lactamase-producing Enterobacteriaceae of water samples in Tunisia. *Science of The Total Environment*, *550*, pp.1103-1109
- Shriram, V., Khare, T., Bhagwat, R., Shukla, R. and Kumar, V., 2018. Inhibiting bacterial drug efflux pumps via phyto-therapeutics to combat threatening antimicrobial resistance. *Frontiers in microbiology*, *9*, p.2990.
- Silveira, R.F., Roque-Borda, C.A. and Vicente, E.F., 2021. Antimicrobial peptides as a feed additive alternative to animal production, food safety and public health implications: An overview. *Animal Nutrition*, *7*(3), pp.896-904.
- The Lancet Infectious Diseases, 2017 Antibiotic research priorities: ready, set, now go. *The Lancet. Infectious diseases*, *17*(4), p.349.
- Vaz, M.S.M., Simionatto, E., de Souza, G.H.D.A., Fraga, T.L., de Oliveira, G.G., Coutinho, E.J., dos Santos, M.V.O. and Simionatto, S., 2022. Zingiber officinale Roscoe essential oil: An alternative strategy in the development of novel antimicrobial agents against MDR bacteria. *Industrial Crops and Products*, *185*, p.115065.
- Vyas, P., Suthar, A., Patel, D., Dayma, P., Raval, J. and Joshi, D., 2015. Antimicrobial activity of extracts of Cinnamomum zeylanicum bark and its combination with antibiotics against various microorganisms. *J Chem Pharm Res*, *7*(4), pp.68-70.
- World Bank (2017). Drug-Resistant Infections: A Threat to Our Economic Future. Available at: https://www.worldbank.org/en/topic/health/publication/ drugresistant-infections-a-threat-to-our-economic-future (Accessed February 12, 2021).
- World Health Organization (2017). WHO Publishes List of Bacteria for Which New Antibiotics Are Urgently Needed. Available at: https://www.who.int/news/ item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics are-urgently-needed (Accessed February 12, 2021).
- Yu, Z., Tang, J., Khare, T. and Kumar, V., 2020. The alarming antimicrobial resistance in ESKAPEE pathogens: Can essential oils come to the rescue?. *Fitoterapia*, *140*, p.104433.