



ANTIBACTERIAL POTENTIAL OF METHANOLIC EXTRACT OF CARDAMOM (*Elettaria cardamomum*) PODS AGAINST MULTIDRUG RESISTANT *Klebsiella pneumoniae* AND *Staphylococcus aureus*

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ABSTRACT

The unprecedented challenges in the treatment of infectious diseases caused by the alarming increase of multidrug resistant bacteria are serious concern worldwide. The present study focused on evaluating the antibacterial activity of methanolic extract of cardamom (*Elettaria cardamomum*) pods; and in finding out the minimum inhibitory concentrations (MICs) against *Klebsiella pneumoniae* strain MDR and *Staphylococcus aureus*. The pod extract was also screened for antioxidant activity. The extract at the concentrations of 500 and 1000 mg mL⁻¹ showed higher zones of growth inhibition against *K. pneumoniae* and *S. aureus*. MICs of *K. pneumoniae* and *S. aureus* were found to be 7.8 and 3.9 mg, respectively, and the antioxidant values were greater (57.95 ± 0.01% of DPPH scavenging and 92.96 ± 0.01% of reducing power) at the concentration of 1000 µg extract. The study revealed that the methanolic pod extract of cardamom pods have significant antibacterial potential against MDR pathogens and can be developed as treatment for MDR infections.

Keywords: Antimicrobial resistance, antioxidant activity, cardamom, minimum inhibitory concentration, multidrug resistance

INTRODUCTION

The potential of microorganisms to develop resistance against commonly used antibiotics have long been recognized. The incidence of antibiotic-resistant bacteria varies substantially with location, nation and susceptible population, since the harshness of problem is carefully related to the efforts made to combat the spread of drug-resistant bacteria (Aslam *et al.*, 2018). For instance, the proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates presently grew from zero (10-15 years ago) to nearly 28% in USA, 30% in UK, 40% in Belgium and 70% in Japan and Republic of Korea (Morrison and Zembower, 2020). Antimicrobial resistance (AMR) is a serious public health concern because it limits the efficacy of treatments in bacterial infections resulting in higher health-care costs, prolonged morbidity and increased mortality. The multidrug resistant (MDR) condition blocks disease control by increasing the spread of resistant microbes and lowering the treatment efficacy, thereby extends the length of infection in patients (Rodríguez-Baño *et al.*, 2021). AMR is a great concern for professionals, governments, etc. and in many countries it has been categorized as a national security hazard (Dadgostar, 2019). The malpractices, mismanagement and extensive use of antimicrobials accelerate the process of anti-microbial resistance. The accessibility to antibacterial drugs without professional monitoring, the usage of drugs with

lesser effectiveness and low potency, easier availability of antibiotics from local shops, poor product quality, etc. are some of the factors leading to the resistance development (Buchy *et al.*, 2020). The resistant microbes may fend off antimicrobial medications resulting in poor treatment, illness persistence and dissemination. Even though the multidrug resistance is a natural phenomenon, the increase in immuno-compromised patients like diabetic patients, HIV-infected patients, patients with organ transplants, severe burns, etc. gives these bacteria an easy access into the body; and thereby facilitate MDR spread (Taneja and Sharma, 2019; Vidovic and Vidovic, 2020). Therefore, there is a need to develop novel drugs for treatment of MDR infections.

Cardamom (*Elettaria cardamomum* Maton), the queen of spices, is an herbaceous, perennial monocot belonging to the family Zingiberaceae and is cultivated all over the South Asia (Anjali *et al.*, 2016). Cardamom seeds have roughly 4% essential oil on dry weight basis with 1,8-cineole as major constituent ($\geq 50\%$), and terpineol, borneol, camphor, limonene, terphenyl acetate, and pinene in minor amounts and is plentiful in iron, protein, and vitamins B & C but less in fat (Zainab *et al.*, 2020; Ashokkumar *et al.*, 2021). Cardamom seeds are used in aromatherapy to promote energy due to their spicy and sweet scent. It is an ayurvedic aphrodisiac and cure for digestive issues, asthma, bronchitis, urinary difficulties and a variety of other human illnesses (Yahyazadeh *et al.*, 2021). Cardamom has widely been studied for its antimicrobial potency (Al Zuhair *et al.*, 1996; Noumi *et al.*, 2018) against many bacterial pathogens, but no report is available on its anti-multidrug resistant bacterial activity. Therefore, the present study was aimed to assess the antibacterial activities of cardamom pods against MDR *K. pneumoniae* and *S. aureus*. DPPH radical scavenging potentials and ferric reducing antioxidant power of cardamom pod-extract were also evaluated.

MATERIALS AND METHODS

Isolation and identification of clinical pathogens

The clinical pathogens were isolated from the in-patients of a tertiary hospital and the isolates were cultivated in appropriate nutrient medium (HiMedia). The isolates were identified by morpho-biochemical analyses and by growing on selective and differential growth media like mannitol salt agar (MSA) and MacConkey agar (Debnath *et al.*, 2018). Yellow colonies on MSA and the characteristic lactose fermenting pink colonies on MacConkey agar confirmed the pathogens as *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Antibiotic susceptibility test (ABST)

Antibiotic susceptibility of pathogens was assessed by standard disk diffusion (Kirby-Bauer antibiotic susceptibility) test protocol (Radji and Fauziah, 2011). The isolated *S. aureus* and *K. pneumoniae* were individually tested against 5 antibiotics viz., ampicillin (25 µg), methicillin (5 µg), amoxicillin (30 µg), tetracycline (30 µg) and streptomycin (30 µg). The resistance pattern was identified by measuring their inhibition zone; size and compared with the zone interpretation chart (CLSI, 2012).

Processing of cardamom pods and extraction of bioactive compounds

Cardamom pods were dried under shade at room temperature, powdered and stored in sterile container for extraction. The powdered cardamom pods (20 g) were placed in a cellulose made porous bag in the 'thimble' of Soxhlet apparatus. Then 100 mL methanol as solvent was added to the extract and the system left as such for 6 h at 60°C. After incubation, the solvent was evaporated and extracts collected and stored in sterile containers.

Antioxidant activity

DPPH radical scavenging assay: Cardamom pod extract was assessed for antioxidant activity on the basis of its ability to donate hydrogen, with stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as per Blois (1958). The extract was adjusted at the concentrations of 100, 250, 500, 750 and 1000 µg

by using methanol and the total volume made to 100 μL . Then 5 mL of 0.1 mM methanol solution of DPPH was mixed and extract kept at 27°C for 20 min. The absorbance of extract was measured at 517 nm using UV-vis spectrophotometer (Shimadzu UV-1800, Japan). The radical scavenging activity of samples were estimated using the following formula:

$$\% \text{ DPPH radical scavenging activity} = (\text{control OD} - \text{sample OD} / \text{control OD}) \times 100$$

Ferric reducing antioxidant power (FRAP) assay: To evaluate the reducing potentials of extract, FRAP assay was performed (Benzie and Strain, 1996). FRAP reagent containing 2.5 mL 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, 2.5 mL 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 300 mM acetate buffer (25 mL, pH 3.6) was prepared and kept at 37°C. The plant sample concentrations of 100, 250, 500, 750 and 1000 μg were prepared using FRAP reagent and water. The reaction mixture was kept at 37°C for 30 min and absorbance (OD values) noted at 593 nm using UV-vis spectrophotometer (Shimadzu UV-1800, Japan). The values were expressed as percentage of reducing power.

Antibacterial activity using well diffusion method

The antibacterial efficacy of cardamom pod extract against the isolated clinical pathogens was assessed by well diffusion method (Soundararajan *et al.*, 2021). For this, the sterile nutrient agar was prepared and poured into Petri-plates. Overnight cultures of test pathogens were taken and 0.1% culture solution of test organisms streaked all over the petri plate with sterile cotton swab by rotating the plate at 60° angle for every streaking. Using a well borer, a 6 mm bore well was made on agar surface of all the NA plates. The wells were then filled with 100 μL sample and agar plates incubated for 48 h at 37°C. After incubation, the plates were observed for zone of growth inhibition.

Evaluation of minimum inhibitory concentration (MIC) of pod extracts

To determine the MICs of cardamom pod extracts, modified dilution method was followed (Chikezie, 2017). Briefly, in sterile test tubes with nutrient broth, the antibiotic gentamicin (diluted into several concentrations viz., 5.0, 6.0, 7.0, 7.5, 9.0, 9.5, 9.8, 10.0 and 15.0 $\mu\text{g mL}^{-1}$) was taken. A loopful (10 μL) of each test bacterial culture (0.5 McFarland standard, Eucast, 2003) was inoculated into the test tubes having 1 mL nutrient broth with various concentrations of gentamicin in it. The tubes were incubated for 24 h at 37°C and observed for turbidity or growth. Then a loopful of broth from every test tube, which did not show any growth, was inoculated into nutrient agar plate. The sterile nutrient broth in equal volumes was added to the test tube cultures and incubated at 37°C for 24 h. After incubation, both the test tubes and agar plates were observed for turbidity or growth with unaided eye (CLSI, 2012). A repeat of DTM with higher dilution (1:10) as a substitute of the double dilution with nutrient broth was also performed.

Statistical analysis

All the above tests were performed in triplicates. All the data statistically analyzed using Graphpad prism software (version 8.0) and were presented as mean $\pm$ standard deviation.

RESULTS AND DISCUSSION

The resistance pattern of test pathogens against the 5 tested antibiotics is shown in Fig. 1. The isolated *Staphylococcus aureus* showed resistance to four antibiotics (methicillin, ampicillin, tetracycline and amoxicillin) while *Klebsiella pneumoniae* showed resistance to all the test antibiotics. The DPPH radical scavenging activity of cardamom pod extracts was evaluated using ascorbic acid as a standard. The 100 μg pod extract showed $16.83 \pm 0.01\%$ DPPH scavenging activity, while such activity for 250, 500, 750 and 1000 μg extract was 27.55 ± 0.02 , 37.24 ± 0.04 , 42.85 ± 0.01 and $57.95 \pm 0.01\%$, respectively (Fig. 2). Similarly, ascorbic acid showed 41.24 ± 0.04 , 65.72 ± 0.01 , 76.05 ± 0.01 , 83.51 ± 0.02 and $87.13 \pm 1.01\%$ DPPH scavenging activity for 100, 250, 500, 750 and 1000 μg . The ferric reducing antioxidant power of cardamom pod extract was assessed using 5 concentrations.

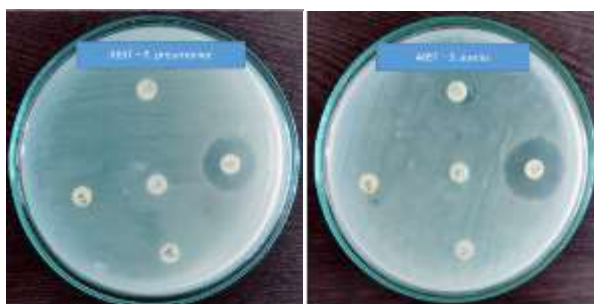


Fig. 1: Antibiotic sensitivity test of the isolated pathogens; *Klebsiella pneumoniae* (left side), *Staphylococcus aureus* (right side)

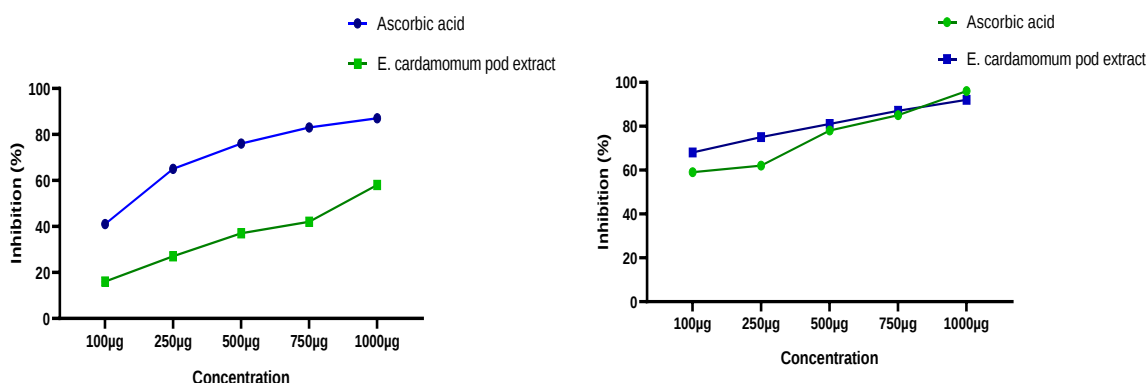


Fig. 2: DPPH radical scavenging activity (left side) and ferric reducing anti-oxidant power (right side) of cardamom pod extracts

zones of growth inhibition were observed in 250 mg mL⁻¹ concentration against either pathogen. The 500 mg mL⁻¹ concentration showed 8.33 ± 0.57 and 11.00 ± 1.00 mm against *S. aureus* and *K. pneumoniae*; while zones of 14.33 ± 0.57 and 15.66 ± 0.57 mm were observed at 1000 mg mL⁻¹ against *S. aureus* and *K. pneumoniae*. The 500 and 1000 mg mL⁻¹ concentration showed higher growth inhibition zones against both test bacteria (Fig. 3 & 4, Table 1).

The minimum concentration of cardamom pod extract showed inhibition against test multidrug resistant pathogens. The concentration showing no pathogenic growth was identified by comparing with positive control. Fig. 5 showed MIC determination against *K. pneumoniae* and *S. aureus*. MICs of cardamom pod extracts against *K. pneumoniae* and *S. aureus* were 7.8 and 3.9 mg, respectively. Methicillin-resistant *S. aureus* (MRSA) infections are widespread in various countries causing mortalities. As per the National Nosocomial Infections Surveillance System, the incidence of nosocomial MRSA infections is gradually increasing in US, and these infections account for >60% critical care unit hospitalizations (Haddadin *et al.*, 2002). Several antimicrobial medicines, including

Table 1: Antibacterial activity of cardamom pod extracts by well diffusion method

Cardamom extract conc. (mg mL ⁻¹)	Inhibitory zones (mm)	
	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>
125	-	-
250	-	7.66 ± 0.57
500	8.33 ± 0.57	11.00 ± 1.00
1000	14.33 ± 0.57	15.66 ± 0.57

second- and third-line medications, have acquired resistance in *S. aureus*. Only a rare medication has been licensed for curing MRSA infections. MRSA's epidemiology is continually evolving, subsequent in an extensive variety of antibiotic resistance patterns across areas and nations. As a result, epidemiologic surveillance is necessary to assist doctors in avoiding and treating the infection. *S. aureus* resistance patterns were gained from blood samples of patients hospitalized to one hospital in

Odisha, eastern India, throughout a two-year period (Wangai *et al.*, 2019; Shrestha *et al.*, 2021).

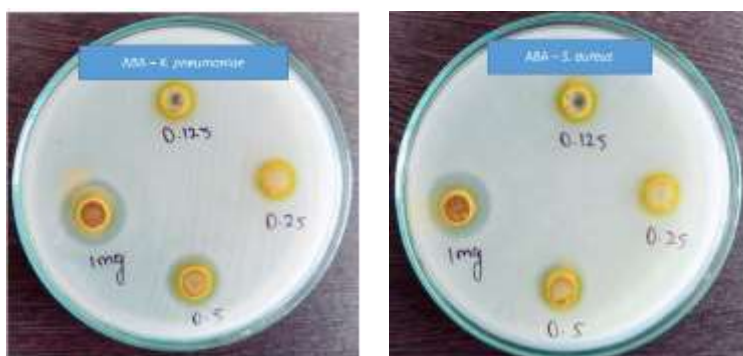


Fig. 3: Antibacterial activity of cardamom pod extract against a) *Klebsiella pneumoniae* b) *Staphylococcus aureus* using well diffusion method

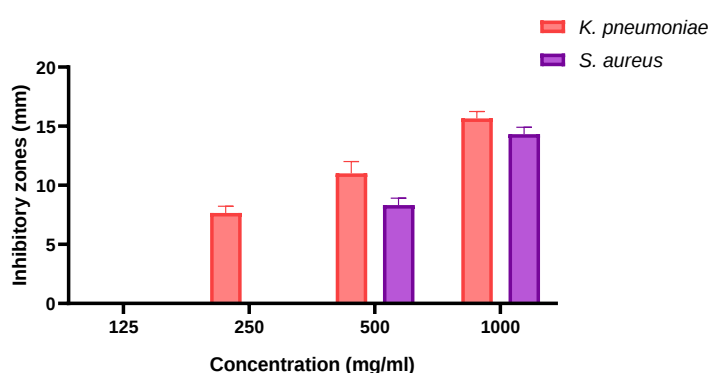


Fig. 4: Antibacterial analysis of cardamom pod extracts against MDR pathogens



Fig. 5: Minimum inhibitory concentration of cardamom pod extracts against *Klebsiella pneumoniae* (left side) and *Staphylococcus aureus* (right side)

et al. (2021) tested the cardamom extract for its *in vitro* anti-oxidant activity utilizing reducing power assay and DPPH radical scavenging. The extract showed DPPH and metal chelating activity with IC₅₀ values of 464 and 199 g mL⁻¹, respectively, while the reducing power and antioxidant activities were 289 AAE mg⁻¹ and 468 GAE mg⁻¹, respectively. This reveals that the extract of this plant has anti-oxidant action. Al Zuhair *et al.* (1996) reported that cardamom oil (280 L kg⁻¹) inhibited 86.4% bacteria, whereas 175 L kg⁻¹ oil inhibited 69.2% bacteria. Oil, on the other hand, has a more effective anti-inflammatory effect than indomethacin at a dosage of 280 L kg⁻¹. Using disc diffusion and microdilution assays, Noumi *et al.* (2018) found significant anti-microbial action of essential oil from cardamom against *Enterococcus faecalis*, *S. aureus* MR (B2), *S. aureus*, *S.*

K. pneumoniae is one of the significant opportunistic bacteria, causing infections in pulmonary system, urinary tract, circulatory system and soft tissues in hospitals and healthcare settings across the world. However, because of its proclivity for developing antibiotic resistance and causing deadly results, *K. pneumoniae* has arisen as a therapeutically relevant microbe in recent two decades. *K. pneumoniae* is recognized as the main reason of community-acquired pneumonia (CAP) in current years, accounting about 10% of all hospital-acquired infections and ranking 2nd among Gram-negative bacteria (Crellen *et al.*, 2019; Wu *et al.*, 2019). Cardamom is traditionally used to treat asthma, gum and teeth infections, kidney and digestive disorders, nausea and diarrhea, constipation, asthma and bronchitis, carminative, cold, cough, pulmonary congestion,

diuretic, kidney disorders, teeth and gum infections, urinary and pulmonary tuberculosis, and irritation of eyelids, bladder infections, constipation, stomach discomfort, and dysentery in children, in the production of various plant-based hand creams and soaps, digestive problems, headaches, and inflammation, and the treatment of colds and their symptoms, it increases vision (Yahyazadeh *et al.*, 2021). Kumar

epidermidis, *Escherichia coli*, *E. coli* ATCC 25922, *Klebsiella pneumoniae* and *Pseudomonas* spp. These results are in line with our findings.

Conclusion: Anti-MDR bacterial activity of cardamom pod against MDR *Klebsiella pneumoniae* and *Staphylococcus aureus* was obvious in the present study. The bioactive compounds of cardamom pods, extracted using methanol as solvent, revealed antibacterial activity and minimum inhibitory concentration against test MDR pathogens. The bioactive compounds from cardamom pod extracts showed significant antibacterial activity against tested MDR pathogens. Apart from the antibacterial analysis other biological properties like anti-oxidant activity was also determined. The pod extracts showed anti-oxidant values on DPPH radical scavenging and reducing power. Further studies are needed to purify and characterize the bioactive compounds responsible for antibacterial activities and accordingly develop novel antibiotics for treatment of MDR infections.

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Conflicts of interest: Authors declare no conflict of interest.

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