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In vitro antimicrobial potency of *Elettaria cardamomum* ethanolic extract against multidrug resistant of food poisoning bacterial strains



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ABSTRACT

Objectives: The antimicrobial resistance of food poisoning strains is considered a public health burden contributing to high mortality and morbidity rates globally. Furthermore, the severe side effects of chemotherapy represent a serious issue which necessitates finding safe sources for anticancer agents for adjuvant therapy to decrease harmful impact of chemotherapeutic drugs. Hence, the current study is conducted to evaluate the antibacterial efficiency of *Elettaria cardamomum* against multidrug resistant strains causing food poisoning outbreaks.

Methods: The resistance profile of the tested food poisoning bacterial pathogens was evaluated using disk diffusion assay. Moreover, the antibacterial efficiency of *E. cardamomum* solvent extracts was achieved using disk diffusion assay to determine the efficiency of these extracts against multidrug resistant strains. Cytotoxicity assay was achieved to assess the antiproliferative efficiency of *Elettaria cardamomum* solvent extracts against MCF7 breast cancer cell line.

Results: Methicillin resistant-*Staphylococcus aureus* (MRSA) strain exhibited resistance to all tested antibiotics except norfloxacin while *E. coli* strain showed susceptibility to all antibiotics used in the study. Furthermore, *P. aeruginosa* showed resistance to cefotaxime, trimethoprim + sulfamethoxazole, cefixime, ceftriaxone and cefaclor antibiotics while it was sensitive to norfloxacin antibiotic. In addition, *S. aureus* strain expressed resistance to ceftazidime, cefixime and ceftriaxone antibiotics. The ethanolic extract of *Elettaria cardamomum* showed the highest antibacterial activity against the concerned food poisoning bacterial strains recording zone diameters ranged from 11.91 ± 0.17 mm to 26.81 ± 0.24 mm. The ethanolic extract of *Elettaria cardamomum* showed MIC values of 0.25 and 0.50 mg/disc against *S. aureus* and *E. coli* strains recording inhibition zone diameters of 16.83 ± 0.14 and 12.34 ± 0.18 mm respectively. GC-MS analysis revealed that α -terpinyl acetate, 1,8-cineole and β pinene were the main phytochemical components of the *Elettaria cardamomum* ethanolic extract recording percentages of 41.24%, 28.14% and 5.98% respectively. In conclusion, the potent efficiency of *E. cardamomum* ethanolic extract against the tested food poisoning strains supports utilizing these extracts in bioformulation of safe and effective food preservatives avoiding side effects of chemical preservatives and controlling the problem of antibiotic resistance.

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Abbreviations: MIC, Minimum inhibitory concentration; MBC, Minimum bactericidal concentration; MHA, Mueller-Hinton Agar; RT, Retention time; M.W, Molecular weight; GC-MS, Gas chromatography-Mass spectrometry.

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1. Introduction

Food poisoning is considered as one of the most common cause of illness and death in developing countries (Abebe et al., 2020). Most epidemiological reports of food poisoning are associated with bacterial contamination, especially members of Gram-negative bacteria such as *E. coli*, *S. typhi* and *P. aeruginosa* (Holmes et al., 2021). In this regard, other Gram positive bacterial pathogens including *Staphylococcus aureus* and MRSA have been reported as causative agents of food borne illness (Rodríguez-Melcón et al., 2021). Antimicrobial resistance is a worldwide issue resulting in microbial tolerance to clinically relevant antibiotics, prompting

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the development of novel treatment options (Frieri et al., 2017). Furthermore, antibacterial resistance poses a serious threat to public health and well-being as the drug resistant bacterial pathogens are thought to cause 2 million illnesses and 23,000 deaths in the United States each year (Hossain et al., 2021). These ailments cost the United States an additional \$20 billion in healthcare costs and \$35 billion of the lost productivity (Abushaheen et al., 2020).

In this regard, the multidrug-resistant *Salmonella* was reported to cause significant number of outbreaks in the U.S (Kumar et al., 2019). On the other hand, the bacterial resistance of *P. aeruginosa* strain represents a significant issue owing to the low permeability of its cell wall, the capability of expressing different resistance mechanisms as overexpression of efflux pumps and porin deletions (Drusano et al., 2021). High incidence of multidrug resistant *P. aeruginosa* strain has been reported in the last few decades causing significant number of mortalities and morbidities globally (Al-Orphaly et al., 2021). Furthermore, *S. aureus* is a Gram positive bacterial strain causing food poisoning illness which can be disseminated through contaminated food (Algammal et al., 2020). Ingestion of food contaminated with toxins of *S. aureus* strain poses staphylococcal food poisoning syndrome resulting in number of symptoms after 1–6 hrs including nausea, gastroenteritis, diarrhea, vomiting and abdominal pain (Bencardino et al., 2021). Methicillin-resistant *S. aureus* (MRSA) has been reported as a prominent nosocomial bacterial pathogen that causes hospitalized infections and has also been associated to foodborne illness (Algammal et al., 2020). Furthermore, MRSA has recently been added to the World Health Organization's list of high-priority drug-resistant pathogens (Dweba et al., 2018). MRSA strains were isolated as contaminants in the food production chain, prompting new research into their transmission among food-producing animals (Tomao et al., 2020). It was discovered that these microbes were found primarily in dairy products such as cheese and milk, as well as meat products such as chicken, rabbit, beef, pork, and turkey (Serra et al., 2021). *Escherichia coli*, on the other hand, is a Gram negative bacterial strain that is normally found as a commensal bacterium in the gut normal flora of human being and other animals (Drider 2021). The pathogenic strains of *E. coli* cause severe food poisoning infections resulting in incidence of severe diarrhea (Gourama 2020). The diarrheal syndrome of *E. coli* infection occurs when the usual net absorptive condition of water and electrolyte absorption is switched to secretion (Butt et al., 2020). Annually, roughly 1.7 billion cases of food poisoning related with diarrheal illnesses are reported worldwide (Bernard and Nicholson 2022). In this sense, diarrhea is the second leading cause of mortality among children under the age of five years (Solomon et al., 2020). Prevention of food deterioration and their causative agent is commonly accomplished by utilizing chemical agents in food preservation (Chaudhari et al., 2020). On the other hand, the frequent use of chemical preservatives was proved to possess harmful side effects on human health resulting from their accumulation in food chain (Hembrom et al., 2020). Furthermore, the overuse of these chemical agents in food preservation results in acquisition of antimicrobial resistance (Rodrigues et al., 2020). Consequently, the high incidence of multi-drug resistant bacterial strains induced by the overuse of antibiotics make it difficult to treat food poisoning illness (Ge et al., 2022). Recently, the use of plant extracts as natural food preservatives has received a great attention due to their safety, effectiveness and easy biodegradability (Jafarzadeh et al., 2020). Plant extracts were reported to be potential source of antimicrobial agents owing to their constituents of phenolic compounds, flavonoids and tannins (Yassin et al., 2021; Yassin et al., 2022a). *Elettaria cardamomum* belongs to Zingiberaceae family and commonly known as green or true cardamom (Ashokkumar et al., 2020). The cardamom essential oil and other recorded bioactive ingredients in cardamom capsules con-

tribute to their unique aroma, nutraceutical and pharmaceutical characteristics (Ozidal et al., 2021). Few studies were performed on the antimicrobial activity of cardamom extracts against multidrug resistant microbes that cause food poisoning illness. In addition, the proven side effects of chemical food preservatives on human health besides the widespread of antimicrobial resistance necessitates the fabrication of natural and safe food preservatives. Hence, the objective of the current study is to detect the antibacterial potency of different *E. cardamomum* solvent extracts against the common etiological agents of food poisoning illness such as *S. aureus*, MRSA, *E. coli*, *P. aeruginosa* and *S. typhimurium*.

2. Materials and methods

2.1. Antibacterial susceptibility testing

Five bacterial strains namely, Methicillin-resistant *S. aureus* (ATCC 43300), *S. aureus* (ATCC 29213), *P. aeruginosa* (ATCC 9027), *S. typhimurium* (ATCC 14023), and *E. coli* (ATCC 25922) were assayed for their susceptibility to different antibiotics. Firstly, freshly prepared bacterial suspension was attained using sterile saline solution (0.85%). Briefly, the bacterial colonies were picked up using sterile loop, immersed in the prepared saline solution and the turbidity was adjusted using 0.5 McFarland standard to attain a viable cell count of 1.0×10^8 cfu/mL. Sterile Mueller-Hinton Agar (MHA) plates were seeded with 0.5 mL of the prepared bacterial suspension. The antibiotic disks used in current study were purchased from MASTDISCS (Mast Group Ltd., Mast House, Merseyside, U.K.). Antibiotic disks namely, cefaclor (CFC), cefazidime (CAZ), cefixime (CFM), cefotaxime (CTX), ceftriaxone (CRO), norfloxacin (NOR) and trimethoprim + sulfamethoxazole (TS) of the concentrations 30, 30, 5, 30, 30, 10 and 25 µg/disk respectively were placed over the seeded plates. The plates were incubated at 37 °C for 24 hrs and sensitivity results were interpreted as stated by the clinical and laboratory standards institute (CLSI 2003) to detect the resistance profile of different tested strains.

2.2. Preparation of *E. Cardamomum* extracts

Elettaria cardamomum seeds were purchased from a local market in Riyadh, Saudi Arabia. The collected plant materials were identified by the herbarium staff of the Botany and Microbiology Department, College of Science, King Saud University. For the extraction of active phytochemicals, four solvents (water, ethanol, ethyl acetate and n-hexane) were utilized in the current study. Firstly, *E. cardamomum* seeds were washed with sterile distilled water after being surface disinfected with 0.5% sodium hypochlorite. The seeds were blinded using a mechanical blender after they had dried completely, and 50 g of the homogenized powder were soaked in 200 mL of various solvents. The flasks were incubated at 25 °C overnight with a magnetic stirrer, then centrifuged at 9000 rpm for 10 min to remove plant residues before being filtered with Whatman filter paper no. 1 to obtain clean filtrates. Finally, using a rotatory evaporator, the clear filtrates were concentrated and stored at 4 °C until use. The extract yields of various solvents were estimated using the formula below:

Extract yield = $(R/S) \times 100$; Where R is the weight of the plant extract residue and S is the raw plant sample weight (Yassin et al., 2020a).

2.3. Screening of antibacterial efficiency of different *E. Cardamomum* extracts

Disc diffusion assay was achieved to detect the susceptibility of the tested food poisoning strains to different seed extracts of *E. car-*

damomum. Sterile filter paper discs (8 mm in diameter) were loaded with 10 mg of different extracts then the discs were placed over MHA plates which were previously seeded with the bacterial suspension as described above. Norfloxacin antibiotic disks (10 µg/disk) were used as positive controls while blank disks, loaded with 50 µl of solvents only without the extracts, were used as negative controls. The dishes were incubated for 24 h at 37 °C, following which the results were determined by measuring the diameter of the inhibitory area using Vernier caliper (Yassin et al., 2022b).

2.4. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The ethanolic extract of *E. cardamomum* was tested for its MIC as this extract showed the highest antibacterial efficiency. MIC was determined for the ethanolic extract of *E. cardamomum* against *S. aureus* and *E. coli* strains exhibiting the highest susceptibility to the extract. 8 mm filter paper discs were loaded with concentrations of 0.125, 0.250, 0.50, 1.00, 2.00, and 4.00 mg/ml then placed over the seeded MHA plates which were previously prepared as described above. The dishes were incubated at 37 °C overnight, then the inhibitory zones were measured using a Vernier caliper, and the MIC was recorded as the lowest concentration showing clear zone formation. To measure MBC, streaks were obtained from suppressive zones of the MIC concentration and subsequent concentrations, inoculated in sterile MHA plates, and incubated overnight at 37 °C. The plates were then examined for bacterial growth, and MBC was defined as the lowest concentration that showed no microbial growth (Yassin et al., 2020b).

2.5. GC–MS analysis of *E. Cardamomum* ethanolic extract

Phytochemical active ingredients of *E. cardamomum* ethanolic extract were instrumentally detected using GC–MS analysis. The instrumental phytochemical analysis was performed using GC–MS Thermo Trace GC Ultra/TSQ Quantum GC equipped with TR5-MS capillary column. The operating analysis conditions were set as previously described by Yassin et al., 2020b. The effective compounds of the *E. cardamomum* ethanolic extract were identified by matching GC–MS results with spectral mass data and retention times of the NIST library.

2.6. Cytotoxicity assay

This cytotoxicity assay is based on the notion that tetrazolium is not reduced by dead cells or their products. The premise is that the mitochondrial enzyme succinate dehydrogenase cleaves the tetrazolium salt MTT into a blue-colored product (formazan). The amount of formazan produced by the cells was found to be related to the number of cells. The MCF7 breast cancer cells (ATCC HTB-22) were subcultured in minimal essential medium supplemented with gentamicin antibacterial agent of 0.1% concentration and 5% fetal calf serum. For cell adherence to the plate, MCF7 cells were injected in 96-well plates and incubated at 37 °C overnight in a 5% CO₂ incubator. The crude seed extracts of *E. cardamomum* were dissolved in methanol (10 mg/mL) and serial dilutions were made accordingly. The extracts were added to the wells containing the adherent cells in doses ranging from 0.0065 to 1 mg/ml. The supernatants were removed after 48 h of treatment, and the developing solution (MTT) was applied at 5 mg/mL concentration to the wells for the aim of formazan crystals formation. The 96-well plates were further incubated at 37 °C for 4 hrs with the supernatants removed. Finally, 50 µl of dimethyl sulfoxide (DMSO) were injected to the wells to stabilize the formazan crystals that had formed. At a wavelength of 570 nm, the absorbance of the soluble formazan in plates was measured using a microplate reader

(Yassin et al., 2020c). The absorbance corresponding to the concentration that inhibits cell viability by 50% (IC₅₀) was estimated according to the following formula:

$$\text{Percentage of inhibition \%} = 100 - \frac{\text{O.D. mean of tested group}}{\text{O.D. mean of Control group}}$$

2.7. Statistical analysis

GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) was used to analyze the data using one-way analysis of variance and Tukey's test. All experiments were done in triplicates, and the results were expressed as mean of triplicates ± standard error.

3. Results and discussion

3.1. Antibiotic susceptibility testing

Antibiotic sensitivity criteria of different tested antibiotics in the study were summarized as seen in Table 1. Methicillin resistant- *Staphylococcus aureus* (MRSA) strain exhibited resistance to all tested antibiotics except norfloxacin while *E. coli* strain showed susceptibility to all antibiotics used in the study. Furthermore, *P. aeruginosa* showed resistance to cefotaxime, Trimethoprim + Sulfamethoxazole, cefixime, ceftriaxone and cefaclor antibiotics while it was sensitive to norfloxacin antibiotic. Furthermore, *S. aureus* strain expressed resistance to cefazidime, cefixime and ceftriaxone antibiotics while *S. typhimurium* strain showed resistance to ceftriaxone only. All the tested bacterial pathogens expressed sensitivity to norfloxacin antibiotic as shown in Table 2 and Fig. 1.

3.2. Extracts yield

The aqueous extract demonstrated the highest productivity yield followed by ethanol, n-Hexane and ethyl acetate solvents recording yield productivity of 9.56%, 7.29%, 6.84% and 4.13% respectively.

3.3. Antimicrobial screening of different *Elettaria cardamomum* solvent extracts against the concerned strains

The ethanolic extract of *E. cardamomum* showed the highest antibacterial proficiency against *S. aureus*, MRSA, *E. coli* and *P. aeruginosa* recording inhibition zone diameters of 26.81 ± 0.24, 24.34 ± 0.12, 22.48 ± 0.36 and 11.91 ± 0.17 mm respectively while *E. cardamomum* ethyl acetate extract recorded the highest bioactivity against *S. typhimurium* strain with suppressive zone diameter of 12.44 ± 0.19 mm as seen in Fig. 2. On the other hand, the aqueous extract of *E. cardamomum* showed no antimicrobial action against *P. aeruginosa* strain.

3.4. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The ethanolic extract of *E. cardamomum* showed the highest antibacterial potency against the tested strains so its MIC was evaluated. *S. aureus* strain exhibited the highest susceptibility to *E. cardamomum* ethanolic extract with MIC value of 0.250 mg/disk demonstrating inhibition zone diameter of 16.83 ± 0.14 mm as seen in Fig. 3. On the other hand, MIC value of *E. cardamomum* ethanolic extract was 0.500 mg/disk with suppressive zone diameter of 12.34 ± 0.18 mm as seen in Table 3. The minimum bactericidal concentrations (MBC) of *E. cardamomum* ethanolic extract

Table 1
Interpretation criteria of the standard antibiotics used in the current study.

Antibiotic agent	Disk potency (µg/disc)	Inhibition zone diameter (mm)		
		Resistant	Intermediate	Sensitive
Cefaclor (CFC)	30	≤14	15–17	≥18
Cefaziodime (CAZ)	30	≤14	15–17	≥18
Cefixime (CFM)	5	≤15	16–18	≥19
Cefotaxime (CTX)	30	≤14	15–22	≥23
Ceftriaxone (CRO)	30	≤24	25–26	≥27
Norfloxacin (NOR)	10	≤12	13–16	≥17
Trimethoprim + Sulfamethoxazole (TS)	25	≤15	16–18	≥19

Table 2
Antibiotic susceptibility data of food poisoning bacterial strains to the standard antibiotics.

Antibiotics (µg/disk)	Inhibition zone diameter (mm)				
	MRSA	<i>P. aeruginosa</i>	<i>S. typhimurium</i>	<i>S. aureus</i>	<i>E. coli</i>
Cefaclor (CFC)	0.00 ± 0.00 (R)	0.00 ± 0.00 (R)	18.76 ± 0.31 (S)	18.75 ± 0.26(S)	20.55 ± 0.22 (S)
Cefaziodime (CAZ)	0.00 ± 0.00 (R)	16.12 ± 0.21 (I)	17.97 ± 0.38 (I)	0.00 ± 0.00 (R)	26.06 ± 0.23 (S)
Cefixime (CFM)	0.00 ± 0.00 (R)	0.00 ± 0.00 (R)	18.91 ± 0.14 (I)	0.00 ± 0.00 (R)	19.55 ± 0.46(S)
Cefotaxime (CTX)	0.00 ± 0.00 (R)	0.00 ± 0.00 (R)	22.45 ± 0.43 (I)	21.17 ± 0.28 (I)	31.61 ± 0.42(S)
Ceftriaxone (CRO)	0.00 ± 0.00 (R)	12.05 ± 0.14 (R)	23.25 ± 0.17 (R)	20.05 ± 0.31(R)	28.17 ± 0.34 (S)
Norfloxacin (NOR)	18.95 ± 0.35 (S)	21.26 ± 0.18 (S)	21.82 ± 0.24 (S)	19.71 ± 0.37 (S)	29.98 ± 0.25(S)
Trimethoprim + Sulfamethoxazole (TS)	15.65 ± 0.26 (I)	0.00 ± 0.00 (R)	18.93 ± 0.45 (I)	18.75 ± 0.29 (I)	28.45 ± 0.42 (S)

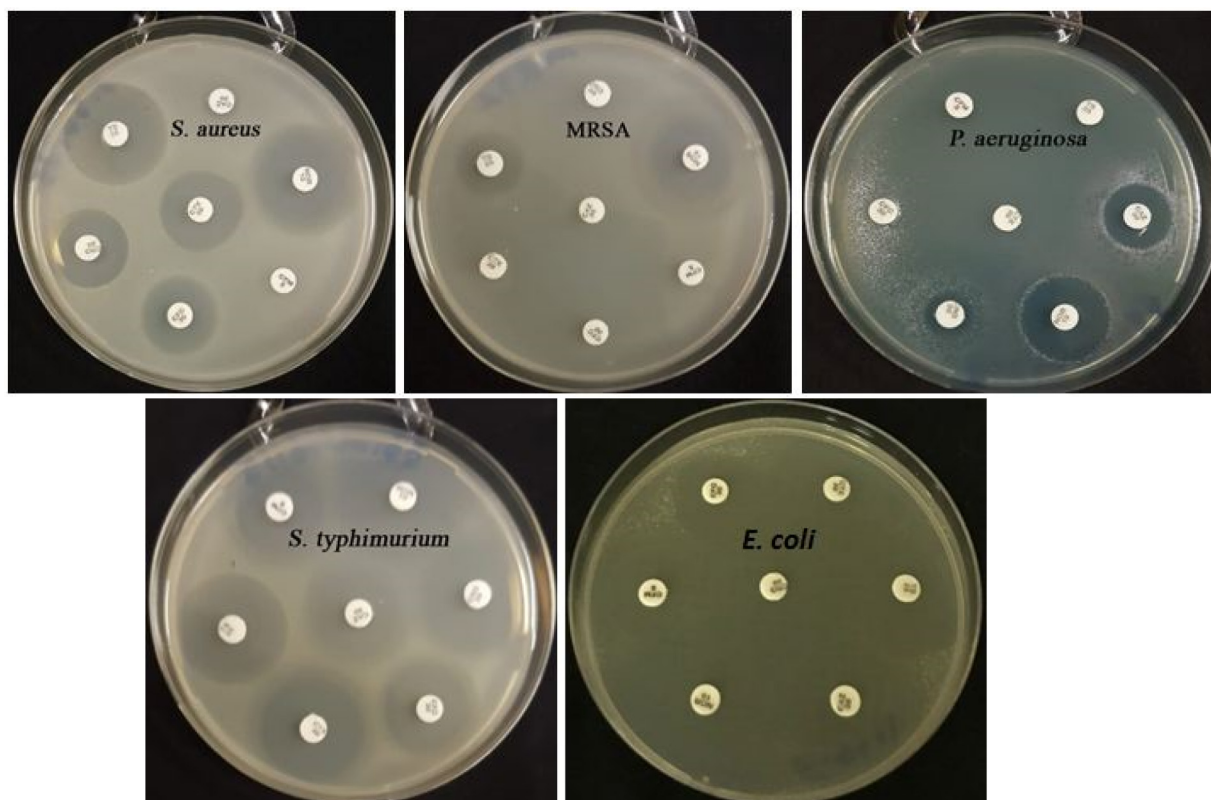


Fig. 1. Antibacterial resistance profile of different food poisoning strains to the concerned standard antibiotics.

against *S. aureus* and *E. coli* were 0.500 and 1.000 mg/disk respectively.

3.5. Phytochemical characterization of *Elettaria cardamomum* ethanolic extract

GC–MS analysis of the ethanolic extract of *E. cardamomum* was performed as this extract exerted the highest antibacterial efficacy against the tested strains. α-Terpinyl acetate (41.24%) was the

major active ingredient of *E. cardamomum* ethanolic extract followed by 1,8-cineole (28.14%), β pinene (5.98%), p-linalool (4.52%), sabinene (4.24%), α-terpineol (3.24%), linalyl acetate (2.78%), myrcene (2.57%), n-hexadecanoic acid (1.87%), a-farnesene (1.46%), p-cymene (1.21%), p-cresol (1.12%), nerol (0.89%) and geranyl acetate (0.65%) as seen in Table 4. The chemical structures of the bioactive components of *E. cardamomum* ethanolic extract exhibiting the highest antibacterial efficiency were shown in Fig. 4.

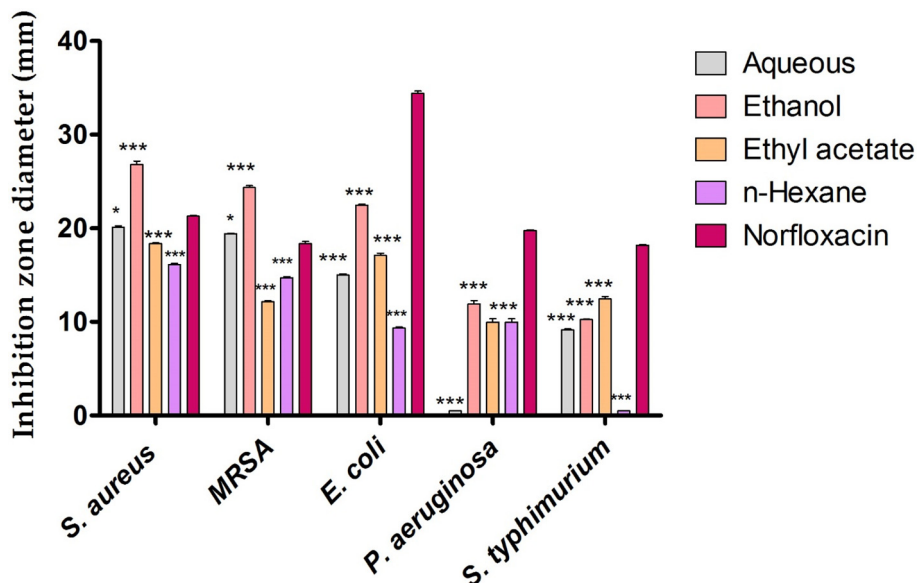


Fig. 2. Antibacterial efficiency of *Elettaria cardamomum* solvent extracts against food poisoning bacterial strains.* Asterisks indicated that values were significantly different compared to control (** $P < 0.001$), (* $P \leq 0.05$).

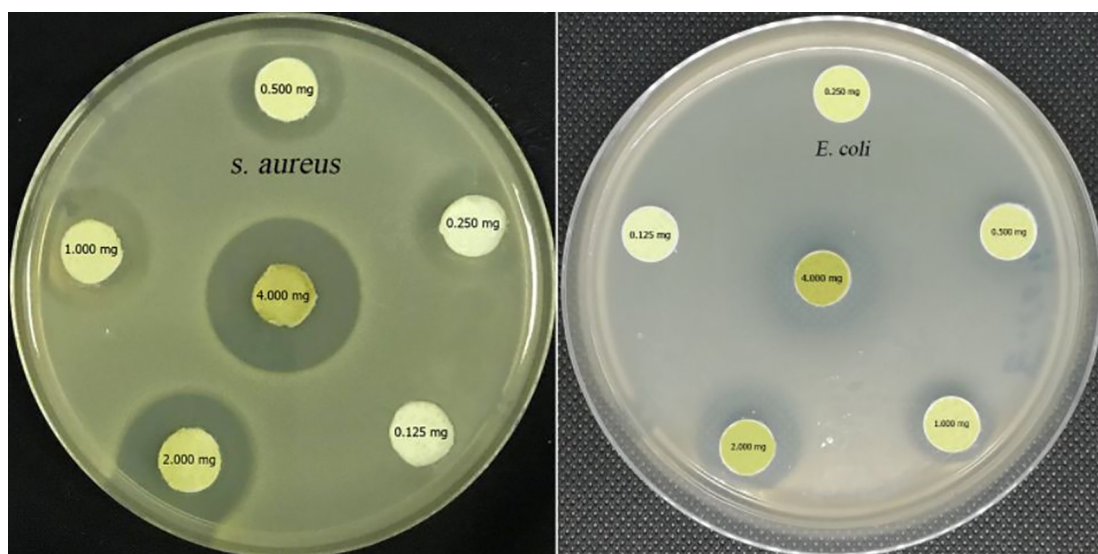


Fig. 3. Minimum inhibitory concentrations of *Elettaria cardamomum* ethanolic extract against *S. aureus* and *E. coli* strains.

Table 3
Minimum inhibitory concentrations of *Elettaria cardamomum* ethanolic extract.

Concentration (mg/disc)	Inhibition zone diameter	
	<i>S. aureus</i>	<i>E. coli</i>
0.125	0.00 ± 0.00	0.00 ± 0.00
0.250	16.83 ± 0.14	0.00 ± 0.00
0.500	17.39 ± 0.35	12.34 ± 0.18
1.000	18.15 ± 0.27	14.89 ± 0.41
2.000	19.24 ± 0.51	18.45 ± 0.23
4.000	20.82 ± 0.46	19.32 ± 0.16

3.6. Cytotoxicity assay

The ethyl acetate extract exhibited the highest cytotoxicity against MCF7 cell line while the aqueous extract showed the lowest cytotoxicity with IC_{50} of 114.8 and 187.9 $\mu\text{g/ml}$ respectively. The ethanolic and hexanic extracts of *E. cardamomum* exhibited

moderate toxicities with IC_{50} of 156.9 and 128.2 $\mu\text{g/ml}$ respectively as seen in Fig. 5.

4. Discussion

Staphylococcus aureus strain has the ability to induce antibiotic resistance owing to its capability to gain antimicrobial resistance as the methicillin resistance via number of mechanisms (Craft et al., 2019). In our current study, the methicillin-resistant *Staphylococcus aureus* (MRSA) exhibited resistance to β -lactam antibiotics as cefaclor which belongs to the second generation cephalosporins. In this sense, β -lactam resistance of MRSA strains represents a serious burden for eradication of *S. aureus* infections (Lee et al., 2018). Cefaclor resistance was found in 91.2% of clinical MRSA isolates, while cefaclor resistance was detected in 25% of strains isolated from healthy individuals (Alharbi 2020). On the other hand, MRSA strain exhibited resistance to third generation

Table 4
Phytochemical active ingredients of *Elettaria cardamomum* ethanolic extract.

Phytochemical constituents	Chemical formula	Mol. weight	RT(min.)	% of Total
β Pinene	C ₁₀ H ₁₆	136.23	4.874	5.98
Sabinene	C ₁₀ H ₁₆	136.23	5.382	4.24
Myrcene	C ₁₀ H ₁₆	136.23	6.257	2.57
1,8-Cineole	C ₁₀ H ₁₈ O	154.25	7.895	28.14
p-Cymene	C ₁₀ H ₁₄	134.21	8.754	1.21
p-Linalool	C ₁₀ H ₁₈ O	154.25	10.357	4.52
α-Terpineol	C ₁₀ H ₁₈ O	154.25	11.218	3.24
Nerol	C ₁₀ H ₁₈ O	154.25	13.385	0.89
Linalyl acetate	C ₁₂ H ₂₀ O ₂	196.29	15.571	2.87
α-Terpinyl acetate	C ₁₂ H ₂₀ O ₂	196.29	16.173	41.24
Geranyl acetate	C ₁₂ H ₂₀ O ₂	196.29	17.945	0.65
a-Farnesene	C ₁₅ H ₂₄	204.36	19.214	1.46
p-Cresol	C ₇ H ₈ O	108.14	21.785	1.12
n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	25.564	1.87

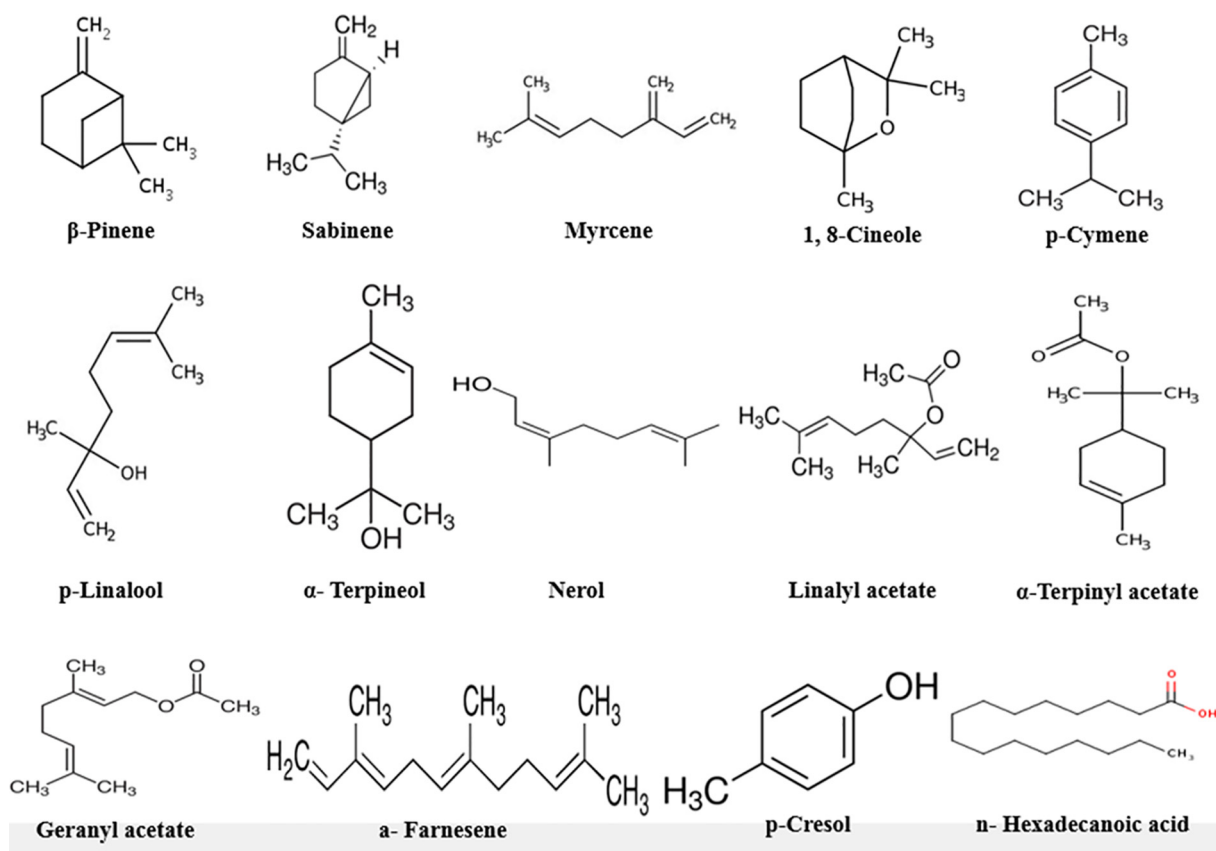
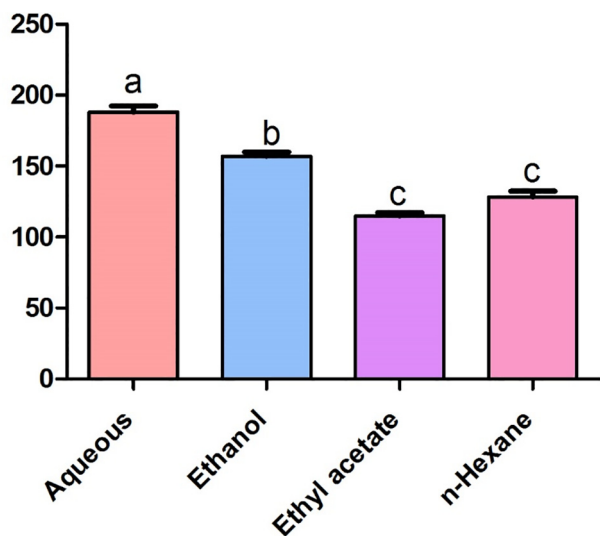


Fig. 4. Phytochemical active components of *Elettaria cardamomum* ethanolic extract.

cephalosporins used in the current study as ceftriaxone, cefazidime, cefixime and cefotaxime antibiotics. Our results were in accordance with that of [Naimi et al., 2017](#) who reported that the clinical isolates of MRSA were less susceptible to cefixim, azithromycin, ceftriaxone, and penicillin antibiotics ([Naimi et al., 2017](#)). Furthermore, [Gashe et al., 2018](#) reported resistance to third-generation cephalosporins such as ceftriaxone and cefazidime in *S. aureus* isolates obtained from Jimma University Specialized Teaching Hospital (JUSH), with resistance percentages of 23.4% and 34% of the total number of isolates, respectively. The expression of the β-lactamase enzyme, which is capable of inactivating antibiotics by hydrolyzing the β-lactam ring, is one of the key mechanisms of β-lactam resistance in *Staphylococci* ([Kim et al., 2020](#)). The other main mechanism of β-lactam resistance is the acquirement of penicillin-binding protein (PBP 2a) gene which

has the potential to remain active in the presence of β-lactam antibiotics so that enables the bacterial growth in presence of β-lactam antibiotics ([Foster 2019](#)). Similarly, *P. aeruginosa* strain showed resistance to the third generation cephalosporins tested in the current study while exhibited an intermediate susceptibility to cefazidime antibiotic. *Pseudomonas aeruginosa*, on the other hand, exhibited resistance to trimethoprim + sulfamethoxazole (co-trimoxazole) antibiotics. These findings were inconsistent with that of [Maclean et al., 2022](#) who reported the sensitivity of all *P. aeruginosa* strains isolated from Sodwana Bay at South Africa to Bactrim (trimethoprim-sulfamethoxazole) antibiotic. The overexpression of multidrug efflux system enhance the intrinsic resistance of *P. aeruginosa* strain to trimethoprim-sulfamethoxazole antibiotics ([Behzadi et al., 2021](#)). *Salmonella typhimurium* exhibited resistance to ceftriaxone antibiotic while showed an intermediate



IC₅₀ of different *E. cardamomum* solvent extracts

Fig. 5. IC₅₀ of different solvent extracts of *Elettaria cardamomum* against MCF7 cell line.* Different letters indicated that values were significantly different ($P \leq 0.05$).

susceptibility to cefazidime, cefixime, cefotaxime and trimethoprim + sulfamethoxazole antibiotics.

The microbiological resistance of the tested food poisoning bacterial strains, such as *S. aureus*, MRSA, *P. aeruginosa*, and *S. typhimurium*, to the antibiotics utilized in this study necessitates the development of novel antimicrobial drugs to address the high rate of antibacterial resistance (Manso et al., 2022). Furthermore, chemical agents employed in food preservation have been linked to a high occurrence of multidrug resistant strains, in addition to the negative effects of these agents on humans and animals, necessitating the development of novel antimicrobial agents (Miethke et al., 2021). To achieve this goal, the antibacterial efficiency of different *E. cardamomum* extracts against food poisoning strains was evaluated. *E. cardamomum* oil is well-known for its distinctive aroma and is frequently utilized as a flavoring and fragrance agent in the food and cosmetic industries (Anwar et al., 2016).

On the other hand, the Gram positive bacterial strain, *S. aureus*, has the capability of releasing staphylococcal enterotoxins in food which are characterized by their high thermostability (Mahros et al., 2021). Moreover, the staphylococcal food poisoning disease is considered one of the main culprits of foodborne outbreaks results in causing severe gastroenteritis (Mourenza et al., 2021). The antibacterial efficacy of the ethanolic extract of *E. cardamomum* against *S. aureus* and the multidrug resistant strain (MRSA) was significantly higher than that of the control (Norfloxacin).

Moreover, *E. coli* is a commensal bacterium that lives in the human colon and frequently causes severe diarrhea (Boxall et al., 2020). *P. aeruginosa*, on the other hand, causes diarrhea in immune-compromised patients and is difficult to relief due to its intrinsic resistance to various medications, whereas *P. aeruginosa* can cause severe gastrointestinal illnesses if it is spread through the food chain (Langendonk et al., 2021; Fakhkhari et al., 2022). *Salmonella typhimurium* is another Gram negative bacterial strain that causes enteric fever disease resulting in gastroenteritis and septicemia (Gal-Mor et al., 2014). The ethanolic extract of *E. cardamomum* was highly efficient against Gram negative microbes, with suppressive zone widths of 11.91 ± 0.17 and 22.48 ± 0.36 mm for *P. aeruginosa* and *E. coli*, respectively. In contrast, *Salmonella typhimurium* expressed the highest sensitivity to *E. cardamomum* ethyl acetate extract at the concentration of 10 mg/disk recording zone diameter of 12.44 ± 0.19 mm. The remarkable efficacy of *E.*

cardamomum extracts against several etiological agents of food poisoning illness demonstrates the potential for employing these extracts in the bioformulation of safe and potentially efficient food preservatives.

Accordingly, the high efficiency of *E. cardamomum* ethanolic extract against majority of the tested food poisoning strains was recorded so its MIC was evaluated. Minimum inhibitory concentrations of *E. cardamomum* ethanolic extract were 0.250 and 0.500 mg/disk against *S. aureus* and *E. coli* strains recording clear zone diameters of 16.83 ± 0.14 and 12.34 ± 0.18 mm respectively. These findings were contrasted with that of Asghar et al., 2017 who reported that MIC value of *E. cardamomum* oil against *S. typhimurium* and *S. aureus* was 10 mg/ml. Another study evaluated MIC of *E. cardamomum* oil against *E. coli* and *P. aeruginosa* strains recording values of 1.0 and 0.5 mg/ml respectively (Alam et al., 2021). Accordingly, the substantial bioactivity of *E. cardamomum* extracts in the current investigation, when compared to previous findings, ensures the extraction procedure's efficiency. A prior study confirmed the antibacterial efficiency of both cardamom seed and fruit extracts against *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia* and *Porphyromonas gingivalis* recording MIC of 0.25% [v/v], 0.5%, 0.125% and 0.062% respectively while MBC were found to be 0.25% [v/v], 1.0%, 0.25% and 0.062% respectively (Souissi et al., 2020). Chemical analysis of the active ingredients of *E. cardamomum* ethanolic extract indicated the presence of three main oxygenated monoterpenes compounds, namely, α -terpinyl acetate, 1,8-cineole and β pinene, recording relative percentages of 41.24, 28.14 and 5.98%, respectively. Our results were coincident with that of Alam et al. (2021b) who reported that α -terpinyl acetate and 1,8-cineole were the main phytochemical compounds of *E. cardamomum* oil (Alam et al., 2021a). Another previous study detected the chemical composition of cardamom essential oil using GC-MS and showed the presence of 26 phytoactive compounds with α -terpinyl acetate (38.4%), 1,8-cineole (28.71%), linalool acetate (8.42%), sabinene (5.21%), and linalool (3.97%) as major phytochemical active constituents (Asghar et al., 2017). These monoterpene compounds were reported to possess antimicrobial effectiveness contributing to the potential bioactivity of *E. cardamomum* extracts (Nazzaro et al., 2013). The antibacterial activity of oxygenated monoterpenes was previously reported to be mediated by their penetration into the fatty acid acyl chains of membrane lipid bilayers, which disrupted cell membrane permeability and fluidity (Tsuchiya 2015). Cytotoxicity assay revealed that the *E. cardamomum* ethyl acetate extract exhibited the highest antiproliferative efficiency while the aqueous extract showed the highest cytotoxic activity against MCF7 cell line recording IC₅₀ of 114.8 and 187.9 μ g/ml respectively. These findings were in accordance with that of Manjunath and Mahurkar 2021 who demonstrated that cardamom oil exhibited a significant anticarcinogenic activity against skin cancer cell line recording IC₅₀ of 166.63 μ g/ml (Manjunath and Mahurkar 2021). The major monoterpene component in *E. cardamomum* extract is terpinyl acetate, which has been shown to have anticancer properties (Chowdhury and Kumar 2020).

5. Conclusion

Antibiotic resistance profile of some etiological agents of food poisoning illness revealed the high resistance of MRSA and *P. aeruginosa* strains while *E. coli* showed susceptibility to all antibiotics used in the current study. The ethanolic extract of *E. cardamomum* exhibited a remarkable antibacterial potency against the tested strains highlighting the potentiality of utilizing these extracts in biofabrication of natural and effective food preservatives. Moreover, the ethyl acetate of *E. cardamomum* extract

showed the highest antiproliferative efficiency against MCF7 cell line indicating the bioefficacy of these extracts in formulation of safe and natural antiproliferative agents. Collectively, Cardamom extracts could be utilized for production of natural, eco-friendly, safe and effective antimicrobial and anticarcinogenic agents owing to their high content of oxygenated monoterpene compounds as demonstrated by GC–MS analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- Abebe, E., Gugsu, G., Ahmed, M., 2020. Review on major food-borne zoonotic bacterial pathogens. *J. Trop. Med.*
- Abushaheen, M.A., Fatani, A.J., Alosaimi, M., Mansy, W., George, M., Acharya, S., Rathod, S., Divakar, D.D., Jhugroo, C., Vellappally, S., Khan, A.A., 2020. Antimicrobial resistance, mechanisms and its clinical significance. *Dis Mon.* 66, (6) 100971.
- Alam, A., Jawaid, T., Alam, P., 2021a. In vitro antioxidant and anti-inflammatory activities of green cardamom essential oil and in silico molecular docking of its major bioactives. *J. Taibah Univ. Sci.* 15 (1), 757–768.
- Alam, A., Rehman, N.U., Ansari, M.N., Palla, A.H., 2021b. Effects of essential oils of *Elettaria cardamomum* grown in India and Guatemala on gram-negative bacteria and gastrointestinal disorders. *Molecules.* 26 (9), 2546.
- Algammal, A.M., Hetta, H.F., Elkesh, A., Alkhalifah, D.H.H., Hozzein, W.N., Batiha, G. E.S., El Nahhas, N., Mabrok, M.A., 2020. Methicillin-Resistant *Staphylococcus aureus* (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. *Infect Drug Resist.* 13, 3255.
- Alharbi, N.S., 2020. Screening of antibiotic-resistant staphylococci in the nasal cavity of patients and healthy individuals. *Saudi J. Biol. Sci.* 27 (1), 100–105.
- Al-Orphaly, M., Hadi, H.A., Eltayeb, F.K., Al-Hail, H., Samuel, B.G., Sultan, A.A., Skariah, S., 2021. Epidemiology of multidrug-resistant *Pseudomonas aeruginosa* in the Middle East and North Africa Region. *Mosphere.* 6 (3), e00202. e00221.
- Anwar, F., Abbas, A., Alkharfi, K.M., 2016. Cardamom (*Elettaria cardamomum* Maton) Oils Essential oils in food preservation, flavor and safety. Elsevier, pp. 295–301.
- Asghar, A., Butt, M.S., Shahid, M., Huang, Q., 2017. Evaluating the antimicrobial potential of green cardamom essential oil focusing on quorum sensing inhibition of *Chromobacterium violaceum*. *J. Food Sci. Technol.* 54 (8), 2306–2315.
- Ashokkumar, K., Murugan, M., Dhanya, M., Warkentin, T.D., 2020. Botany, traditional uses, phytochemistry and biological activities of cardamom [*Elettaria cardamomum* (L.) Maton]—A critical review. *J. Ethnopharmacol.* 246, 112244.
- Behzadi, P., Baráth, Z., Gajdác, M., 2021. It's not easy being green: a narrative review on the microbiology, virulence and therapeutic prospects of multidrug-resistant *Pseudomonas aeruginosa*. *Antibiotics.* 10 (1), 42.
- Bencardino, D., Amagliani, G., Brandi, G., 2021. Carriage of *Staphylococcus aureus* among food handlers: An ongoing challenge in public health. *Food Control.* 130, 108362.
- Bernard, R., Nicholson, M., 2022. Bacterial Infections of the Small and Large Intestine. *Textbook of Pediatric Gastroenterology, Hepatology and Nutrition.* Springer, pp. 203–218.
- Boxall, M.D., Day, M.R., Greig, D.R., Jenkins, C., 2020. Antimicrobial resistance profiles of diarrhoeagenic *Escherichia coli* isolated from travellers returning to the UK, 2015–2017. *J. Med. Microbiol.* 69 (7), 932–943.
- Butt, S., Saleh, M., Gagnon, J., 2020. Impact of the *Escherichia coli* heat-stable enterotoxin b (STb) on gut health and function. *Toxins.* 12 (12), 760.
- Chaudhari, A.K., Singh, V.K., Das, S., Singh, B.K., Dubey, N.K., 2020. Antimicrobial, aflatoxin B1 inhibitory and lipid oxidation suppressing potential of anethole-based chitosan nanoemulsion as novel preservative for protection of stored maize. *Food Bioproc Tech.* 13 (8), 1462–1477.
- Chowdhury, S., Kumar, S., 2020. Alpha-terpinyl acetate: A natural monoterpeneoid from *Elettaria cardamomum* as multi-target directed ligand in Alzheimer's disease. *J. Funct. Foods.* 68, 103892.
- Craft, K.M., Nguyen, J.M., Berg, L.J., Townsend, S.D., 2019. Methicillin-resistant *Staphylococcus aureus* (MRSA): antibiotic-resistance and the biofilm phenotype. *MedChemComm.* 10 (8), 1231–1241.
- Drider, D., 2021. Gut Microbiota Is an Important Source of Bacteriocins and Their In Situ Expression Can Be Explored for Treatment of Bacterial Infections. *Probiotics Antimicrob. Proteins.* 13 (6), 1759–1765.
- Drusano, G.L., Bonomo, R.A., Marshall, S.M., Rojas, L.J., Adams, M.D., Mojica, M.F., Kreiswirth, B.N., Chen, L., Mchledlidze, N., Bacci, M., Vicchiarelli, M., 2021. Emergence of Resistance to Ceftazidime-Avibactam in a *Pseudomonas aeruginosa* Isolate Producing Derepressed bla PDC in a Hollow-Fiber Infection Model. *Antimicrob. Agents Chemother.* 65 (6), e00124–e00221.
- Dweba, C.C., Zishiri, O.T., El Zowalaty, M.E., 2018. Methicillin-resistant *Staphylococcus aureus*: livestock-associated, antimicrobial, and heavy metal resistance. *Infect Drug Resist.* 11, 2497.
- Fakhkhari, P., Tajeddin, E., Azimirad, M., Salmazadeh-Ahrabi, S., Abdi-Ali, A., Nikmanesh, B., Eshtrati, B., Gouya, M.M., Owlia, P., Zali, M.R., Alebouyeh, M., 2022. Involvement of *Pseudomonas aeruginosa* in the occurrence of community and hospital acquired diarrhea, and its virulence diversity among the stool and the environmental samples. *Int. J. Environ. Health Res.* 32 (1), 61–71.
- Foster, T.J., 2019. Can β -lactam antibiotics be resurrected to combat MRSA? *Trends Microbiol.* 27 (1), 26–38.
- Frieri, M., Kumar, K., Boutin, A., 2017. Antibiotic resistance. *J. Infect. Public Health.* 10 (4), 369–378.
- Gal-Mor, O., Boyle, E.C., Grassl, G.A., 2014. Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella enterica* serovars differ. *Front. Microbiol.* 5, 391.
- Gashe, F., Mulisa, E., Mekonnen, M., Zeleke, G., 2018. Antimicrobial Resistance Profile of Different Clinical Isolates against Third-Generation Cephalosporins. *J. Pharm.* 2018.
- Ge, H., Wang, Y., Zhao, X., 2022. Research on the drug resistance mechanism of foodborne pathogens. *Microb. Pathog.* 162, 105306.
- Gourama, H., 2020. Foodborne pathogens Food safety engineering. Springer, pp. 25–49.
- Hembrom, S., Singh, B., Gupta, S.K., Nema, A.K., 2020. A comprehensive evaluation of heavy metal contamination in foodstuff and associated human health risk: a global perspective Contemporary environmental issues and challenges in era of climate change. Springer, pp. 33–63.
- Holmes, C.L., Anderson, M.T., Mobley, H.L., Bachman, M.A., 2021. Pathogenesis of gram-negative bacteremia. *Clin. Microbiol. Rev.* 34 (2), e00234. e00220.
- Hossain, M., Attia, Y., Ballah, F.M., Islam, M., Sobur, M., Levy, S., Rahman, A., Nishiyama, A., Hassan, J., Rahman, M., 2021. Zoonotic Significance and Antimicrobial Resistance in *Salmonella* in Poultry in Bangladesh for the Period of 2011–2021. *Zoonotic Dis.* 1 (1), 3–24.
- Jafarzadeh, S., Jafari, S.M., Salehabadi, A., Nafchi, A.M., Kumar, U.S.U., Khalil, H.A., 2020. Biodegradable green packaging with antimicrobial functions based on the bioactive compounds from tropical plants and their by-products. *Trends Food Sci Technol.* 100, 262–277.
- Kim, S.W., Lee, J.S., Park, S.B., Lee, A.R., Jung, J.W., Chun, J.H., Lazarte, J.M.S., Kim, J., Seo, J.S., Kim, J.H., Song, J.W., 2020. The importance of porins and β -lactamase in outer membrane vesicles on the hydrolysis of β -lactam antibiotics. *Int. J. Mol. Sci.* 21 (8), 2822.
- Kumar, D., Pornsukarom, S., Thakur, S., 2019. Antibiotic usage in poultry production and antimicrobial-resistant *Salmonella* in poultry *Food Safety in Poultry Meat Production.* Springer, pp. 47–66.
- Langendonk, R.F., Neill, D.R., Fothergill, J.L., 2021. The building blocks of antimicrobial resistance in *Pseudomonas aeruginosa*: implications for current resistance-breaking therapies. *Front. Cell. Infect. Microbiol.* 11, 307.
- Lee, A.S., De Lencastre, H., Garau, J., Kluytmans, J., Malhotra-Kumar, S., Peschel, A., Harbarth, S., 2018. Methicillin-resistant *Staphylococcus aureus*. *Nat. Rev. Dis. Primers.* 4 (1), 1–23.
- Macleane, K., Njamo, F.O.J.P., Serepa-Dlamini, M.H., Kondiah, K., Green, E., 2022. Antimicrobial Susceptibility Profiles among *Pseudomonas aeruginosa* Isolated from Professional SCUBA Divers with Otitis Externa, Swimming Pools and the Ocean at a Diving Operation in South Africa. *Pathogens.* 11 (1), 91.
- Mahros, M.A., Abd-Elghany, S.M., Sallam, K.I., 2021. Multidrug-, methicillin-, and vancomycin-resistant *Staphylococcus aureus* isolated from ready-to-eat meat sandwiches: An ongoing food and public health concern. *Int. J. Food Microbiol.* 346, 109165.
- Manjunath, C., Mahurkar, N., 2021. In vitro cytotoxicity of cardamom oil, lemon oil, and jasmine oil on human skin, gastric, and brain cancer cell line. *J. Cancer Res. Ther.* 17 (1), 62.
- Manso, T., Lores, M., de Miguel, T., 2022. Antimicrobial Activity of Polyphenols and Natural Polyphenolic Extracts on Clinical Isolates. *Antibiotics.* 11 (1), 46.
- Miethe, M., Pieroni, M., Weber, T., Brönstrup, M., Hammann, P., Halby, L., Arimondo, P.B., Glaser, P., Aigle, B., Bode, H.B., Moreira, R., 2021. Towards the sustainable discovery and development of new antibiotics. *Nat. Rev. Chem.* 5 (10), 726–749.
- Mourzena, Á., Gil, J.A., Mateos, L.M., Letek, M., 2021. Novel Treatments and Preventative Strategies Against Food-Poisoning Caused by *Staphylococcal* Species. *Pathogens.* 10 (2), 91.
- Naimi, H.M., Rasekh, H., Noori, A.Z., Bahaduri, M.A., 2017. Determination of antimicrobial susceptibility patterns in *Staphylococcus aureus* strains recovered from patients at two main health facilities in Kabul, Afghanistan. *BMC Infect. Dis.* 17 (1), 1–7.
- Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., De Feo, V., 2013. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals.* 6 (12), 1451–1474.

- Ozdal, T., Tomas, M., Toydemir, G., Kamiloglu, S., Capanoglu, E., 2021. Introduction to nutraceuticals, medicinal foods, and herbs Aromatic Herbs in Food. Elsevier, pp. 1–34.
- Rodrigues, G. L., Panzenhagen, P., Ferrari, R. G., Dos Santos, A., Paschoalin, V. M. F., Conte-Junior, C. A. 2020. Frequency of antimicrobial resistance genes in *Salmonella* From Brazil by in silico whole-genome sequencing analysis: An overview of the last four decades. *Front. Microbiol.* 1864.
- Rodríguez-Melcón, C., Alonso-Hernando, A., Riesco-Peláez, F., García-Fernández, C., Alonso-Calleja, C., Capita, R., 2021. Biovolume and spatial distribution of foodborne Gram-negative and Gram-positive pathogenic bacteria in mono- and dual-species biofilms. *Food Microbiol.* 94, 103616.
- Serra, V., Salvatori, G., Pastorelli, G., 2021. Dietary polyphenol supplementation in food producing animals: Effects on the quality of derived products. *Animals.* 11 (2), 401.
- Solomon, E.T., Robele, S., Kloos, H., Mengistie, B., 2020. Effect of household water treatment with chlorine on diarrhea among children under the age of five years in rural areas of Dire Dawa, eastern Ethiopia: a cluster randomized controlled trial. *Infect. Dis. Poverty.* 9 (1), 1–13.
- Souissi, M., Azelmat, J., Chaieb, K., Grenier, D., 2020. Antibacterial and anti-inflammatory activities of cardamom (*Elettaria cardamomum*) extracts: Potential therapeutic benefits for periodontal infections. *Anaerobe.* 61, 102089.
- Tomao, P., Pirolo, M., Agnoletti, F., Pantosti, A., Battisti, A., Di Martino, G., de Araujo, F.P., 2020. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* from dairy farms in North-eastern Italy. *Int. J. Food Microbiol.* 332, 108817.
- Tsuchiya, H., 2015. Membrane interactions of phytochemicals as their molecular mechanism applicable to the discovery of drug leads from plants. *Molecules.* 20 (10), 18923–18966.
- Yassin, M.T., Mostafa, A.-A.-F., Al-Askar, A.A., 2020a. In vitro anticandidal potency of *Syzygium aromaticum* (clove) extracts against vaginal candidiasis. *BMC complement. med. ther.* 20 (1), 1–9.
- Yassin, M.T., Mostafa, A.-A.-F., Al-Askar, A.A., 2020b. Anticandidal efficiency of *Cinnamomum zeylanicum* extracts against vulvovaginal candidiasis. *Curr. Sci.* 118 (5), 796.
- Yassin, M.T., Mostafa, A.A., Al-Askar, A.A., 2020c. Anticandidal and anti-carcinogenic activities of *Mentha longifolia* (Wild Mint) extracts in vitro. *J. King Saud Univ. Sci.* 32 (3), 2046–2052.
- Yassin, M.T., Mostafa, A.A.F., Al-Askar, A.A., 2021. In Vitro Evaluation of Biological Activities and Phytochemical Analysis of Different Solvent Extracts of *Punica granatum* L. (Pomegranate) Peels. *Plants* 10 (12), 2742.
- Yassin, M.T., Mostafa, A.A.F., Al-Askar, A.A., Sayed, S.R., 2022a. In vitro antimicrobial activity of *Thymus vulgaris* extracts against some nosocomial and food poisoning bacterial strains. *Process Biochem* 115, 152–159.
- Yassin, M.T., Mostafa, A.A.F., Al-Askar, A.A., Al-Otibi, F.O., 2022b. Facile green synthesis of silver nanoparticles using aqueous leaf extract of *Origanum majorana* with potential bioactivity against multidrug resistant bacterial strains. *Crystals* 12 (5), 603.