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Original article

Evaluation of the Antibacterial Activity of *Zingiber officinale* Extract against *Enterobacter*

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Abstract
Zingiber officinale (ginger) is a medicinal herb known for its bioactive components with potential antibacterial properties. This study evaluates the antibacterial activity of aqueous and ethanolic extracts of ginger, sourced from Diyala, Iraq, against *Enterobacter* species. Extracts were prepared and tested in varying concentrations at the laboratories of the Department of Biology, College of Science, Diyala University. The results showed that *Enterobacter* was resistant to the aqueous extract at a concentration of 0.1 mg/mL. However, at 0.2 mg/ml, the bacteria showed sensitivity, with an inhibition zone of 2 cm, while o.3mg/mL resulted in a 5 cm inhibition zone. In contrast, the ethanolic extract demonstrated higher antibacterial activity: although *Enterobacter* remained resistant at 0.1 mg/mL, it was sensitive at 0.2 mg/mL (5 cm inhibition zone), and the inhibition zone increased to 6 cm at 0.3 mg/mL. Overall, ethanolic extracts exhibited greater antibacterial effectiveness than aqueous extracts. The antibacterial activity of ginger is influenced by factors such as extract concentration, extraction method, and bacterial strain type. While conventional antibiotics generally show higher effectiveness, ginger extracts may serve as a natural antibacterial agent, particularly in mild or supportive clinical applications. Based on these findings, ginger has potential as both a flavoring agent and a natural antibacterial compound.
Keywords. *Zingiber* officinale, Ginger, *Enterobacter*, Antibacterial Activity.

Introduction

The genus *Enterobacter* comprises facultatively anaerobic Gram-negative bacilli that are rod-shaped and around 2 mm in length. It is characterized as not producing spores, lactose-fermenting, and urease-positive. The virulence of this bacterium is contingent upon a variety of circumstances. Similar to other gram-negative enteric bacilli, the bacteria employ adhesins to adhere to the cells of the host. The presence of a lipopolysaccharide (LPS) capsule enables bacteria to evade opsonophagocytosis. *Capsules* of LPS may initiate an inflammatory process within the host's cell and could ultimately result in sepsis [1]. *Enterobacter* used flagella to move and is classified under the *Enterobacter*iaceae family. Although it was initially characterized in the 1960s, taxonomy has changed in the past half-century [2]. It is possible to obtain a diverse range of types of *Enterobacter* from a variety of sources, including animals, natural settings, and hospitals [3]. The World Health Organization (WHO) has categorized the *Enterobacteriaceae* family as one of the most critical families of bacteria that are resistant to antibiotics [4].

The capacity of the genus *Enterobacter spp.* to endure diverse environmental circumstances results in the emergence of various illnesses in healthcare environments. These pathogenic microbes primarily belong to the respiratory and gastrointestinal microbiome in humans as well as animals. These bacteria facilitate glucose fermentation, resulting in gas and acid generation [5]. Most strains of *Enterobacteriaceae* exhibit a negative methyl red test, while a positive Voges-Proskauer reaction. They also demonstrate positive nitrate reduction to nitrite and alkaline reactions in malonate broth and Simmons citrate. Nevertheless, no selective media exists for *Enterobacter* spp. Typically, these organisms are linked to contamination from blood products, intravenous fluids, cotton swabs, colonized hands of healthcare professionals, and stethoscopes [1]. The *Enterobacter* genus, part of the famous pathogenic group ESKAPE (*Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecium, Klebsiella pneumoniae, Acinetobacter baumannii, and Enterobacter species*), poses an important global concern for the health of humans [6]. *Enterobacteria* can cause many diseases, including intra-abdominal, bloodstream, gastrointestinal, lung, and infection of urinary tract infections, potentially leading to life-threatening bacteremia with a death rate of as much as 45% [7]. The resistance to antibiotics is an escalating issue for the treatment of *Enterobacter* infections. Potential therapies include beta-lactams, carbapenems, sulfamethoxazole/trimethoprim, fluoroquinolones, and aminoglycosides [2].

Medicinal plants have been employed for generations for their therapeutic advantages. Research from the WHO indicates that over 85% of the global population depends predominantly on traditional medicines utilizing plant extracts or their active ingredients [8]. These plants are crucial to traditional healthcare systems for the treatment of various ailments. The therapeutic characteristics of these plants arise from individual chemical compounds that elicit distinct physiological responses [9]. *Zingiber officinale,* generally referred to as "ginger," was a perennial herb that belongs to the Zingiberaceae family [10]. Ginger is the subterranean rhizome characterized by a striated, hard feel. Ginger is employed as an herbal remedy to avert and address numerous ailments, *including* stomach upsets, nausea, diarrhea, and heart problems

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[11]. It is also used to decrease joint pain from arthritis, is useful for the treatment of lung and heart diseases, and relieves colds, cough, and throat infections [12]. In addition to its use as a powder and in hot drinks like ginger tea, ginger has been recognized as an herbal medicinal product with pharmacological effects [13].

Many research investigations have demonstrated that ginger extracts exhibit antibacterial activity and antioxidant properties [14]. Ginger contains volatile oils at a rate between 2.5-3%. They also contain phenolic compound called "gingerols" Which gives it a bitter and spicy taste. Gingerols can be categorized into zingerones, gingerols, shogaols, and paradols. Among these, gingerols and shogaols are recognized as the most significant active physiological components of ginger [15]. These components are regarded as an antidote to Thrombosis, as well as an anti-inflammatory of various conditions, including inflammatory diseases such as asthma, joints, colitis, and migraine [16]. This study explores the extraction of Iraqi ginger and analyzes the antimicrobial activity of its extracts against *Enterobacter*, aiming to provide deeper insights and evidence regarding the bioactivity of Iraqi ginger.

Materials and Methods

Collection of plant materials and processing of samples

Ginger was procured at a local marketplace in Baqubah, Diyala Governorate, Iraq. The material was thoroughly cleaned with water and was air-dried at ambient temperature for four weeks. Pulverization was then carried out using an electric grinder, and the material was stored in a glass jar until it was required for extraction.

Extraction Procedure

Aqueous extraction: Eighty grams of ginger powder (particle size 149 µm) is weighed. The particle size was obtained by sieving the powder through a stainless-steel mesh (149µm, Retsch, Germany) calibrated according to ASTM standards. 250 ml of sterile distilled water is added to the powder. The mixture is stirred continuously with a vibrator for 24 hours at 25°C. The solution is then filtered through filter paper. The filtrate is subsequently stored in a sterile glass bottle in a refrigerator [17].

Alcoholic extraction (Ethanolic extraction): Eighty grams of ginger powder (particle size 149 µm) weighed, and 250 ml of ethanol (70%) was added to the powder. The solution was mixed under continuous stirring with a vibrator for 24 hours at 25°C. The solution is filtered using filter paper. The filtrate is stored in a refrigerator in a sterile glass bottle (the alcoholic extract was prepared by having water replaced with ethanol (70%) in the same procedure as before) or evaporated to dryness to obtain the crude extract. Concentrations of 0.1, 0.2, and 0.3 mg/mL were selected based on preliminary screening results, which indicated these were the most effective ranges. Higher or lower concentrations showed reduced activity or solubility limitations. prepared from the stock solutions of both extracts [18].

Utilized isolates

An isolate of *Enterobacter*-type pathogenic bacteria was obtained and identified in the labs of Baqubah Teaching Hospital.

Bacterial diagnosis

Phenotypic examination

MacConkey agar and blood agar medium were used for culturing, and the cultural characteristics, including colony shape and color, were diagnosed [19].

Biochemical tests

The IMViC tests represent a group of four biochemical assays used to differentiate among members of the family Enterobacteriaceae. These tests include: Indole Test (I): Determines the ability of the organism to convert tryptophan into indole using the enzyme tryptophanase. Methyl Red Test (M): Assesses the production of stable acid end-products from glucose fermentation. Voges-Proskauer Test (V): Detects the production of acetoin, a neutral end-product of glucose fermentation. Citrate Utilization Test (C): Evaluates the ability of the microorganism to use citrate as the sole carbon source.

Catalase Test

A portion of the bacterial culture was transferred to a clean glass slide using wooden sticks, and a few drops of hydrogen peroxide (H_2O_2) reagent were added to it. After that, oxygen gas bubbles appeared, indicating that the test was positive [20].

Oxidase Test

We transfer one of the colonies growing on MacConkey agar to a filter paper, then put 2-3 drops of oxidase reagent over the colony and mix it with the colony using sterile wooden sticks. The appearance of the purple color within 20-30 seconds indicates that the test is positive [21].

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Diagnosis with VITEK2

The VITEK2 microscope from Biome Ruin Company was used to perform a biochemical test for bacterial isolates. This device includes 48 biochemical tests used to diagnose bacteria, and antibiotic sensitivity testing can be performed with this device [22].

Evaluation of antimicrobial efficacy (Agar well diffusion method)

Recent colonies of microorganisms had been emulsified in normal saline, calibrating the turbidity to correspond with the 0.5 McFarland standard of barium sulfate solution (equal to $1x10^6$ CFU/mL). Bacterial lawns were established on sterile Muller-Hinton Agar plates by a uniform streaking technique across the surface of the medium.

Following a 15-minute incubation of the prepared bacterial lawns at ambient temperature, three apertures were created in each plate of agar using a sterilized cork borer. Subsequently, 0.1 ml of both aqueous and ethanolic extracts at concentrations of 0.1, 0.2, and 0.3 mg/ml were allocated into the corresponding wells. Both extracts were tested against *Enterobacter* at the three concentrations aforementioned. A 30-minute pre-diffusion period was allowed before incubating the plates at 37 °C for 24 hours. The zones of inhibition have been determined using a ruler [23].

Antibiotic susceptibility testing for Enterobacter

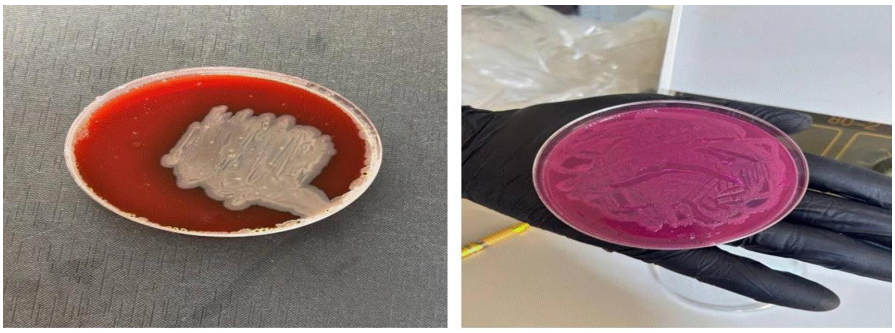
Antibiotic susceptibility testing for Enterobacter was done by the Kirby-Bauer Disk Diffusion Method [24]. Prepare a suspension of bacteria with a 0.5 McFarland titration using Muller-Hinton Agar medium. Use a sterile swab to spread bacteria evenly over the surface of the medium. Insert the appropriate antibiotic tablets (Cephalexin, cephalothin, ceftazidime, Ticarcillin, Piperacillin, and Cefaclor), which were selected due to their clinical relevance and frequent use in treating *Enterobacter* infections. Incubate the dish at 35-37°C for 16-18 hours. Assess the diameter of the inhibitory zones surrounding the discs and analyze the findings in accordance with the CLSI (Clinical and Laboratory Standards Institute) guidelines.

Results and Discussion

Isolation

Ten bacterial isolates were isolated from a total of 88 samples collected from various clinical sources (ear swabs, wounds, burns, and urine samples). The swabs and samples were collected from Baqubah Teaching Hospital and the consulting clinic from October 2024 to February 2025 from hospitalized patients of both sexes and of various ages.

Colonies are often mucoid due to capsule production and appear round, smooth, and slightly raised, some of which showed viscosity and a characteristic odor and were fermented for lactose sugar, while they appeared on the solid blood medium alpha hemolysin [23].



A **B**

Figure (1). Enterobacter on (A) blood agar (B) MacConkey agar

Biochemical test

Biochemical tests for *Enterobacter* help identify them and their team from other members of the *Enterobacteriaceae* family. Here is a summary of the most important tests and their typical results (Table 1) and (Figure 2).

Table (1). Biochemical test for Enterobacter

Biochemical test	Result	Biochemical test	Result
Gram stain	Gram-negative rod	Urease	Variable
Oxidase test	_	H2S production	_

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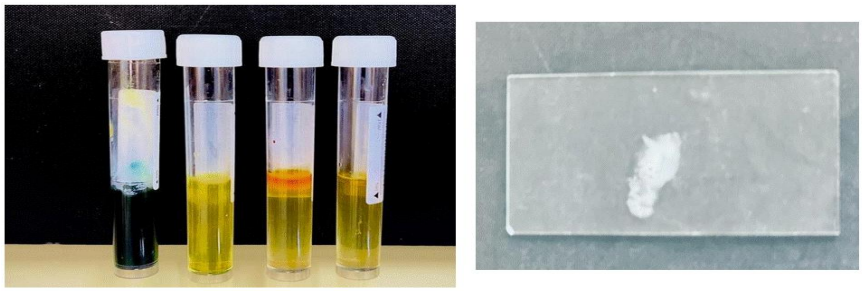
Catalase test	+	Gas production from glucose	+
Indole test	_	Lactose fermentation	+
Methyl Red	_	Motility	Motile
Voges -Proskauer	+	Ornithine decarboxylase	+
Citrate utilization	+	Lysine decarboxylase	Variable



A B

Figure (2). (A) IMVC test (B) catalase test

Inhibitory activity of aqueous extract

The aqueous extract did not show any activity against *Enterobactor* bacteria at this concentration (0.1mg / ml), while at the concentration (0.2mg/ ml) the diameter of inhibition was (2cm), while the concentration (0.3mg/ml) The inhibition diameter was (5cm), and the results are displayed in both (Table 2) and (Figure 3).

Table 2. Type of bacteria and concentrations of the aqueous extract of ginger used the inhibitory effectiveness

Bacteria	0.1 mg/ml	0.2 mg/ml	0.3 mg/ ml
Enterobacter	Resistant	2 cm (Sensitive)	4 cm (Sensitive)

Inhibitory activity of alcoholic extract

The alcoholic extract showed effectiveness against *Enterobacter* bacteria at a concentration of (0.2mg/ml). The diameter of inhibition was (5cm), while the concentration (0.3mg/ml) was the diameter of inhibition (6cm). At a concentration of (0.1mg /ml), no inhibitory activity on bacteria was observed, as shown in (Table 4) and (Figure 3). To ensure that the antibacterial activity was due to the ginger extract and not the alcohol itself, a negative control group using ethanol only was included. No inhibition zone was observed in the control, confirming that the effect is attributed to the ginger extract.

Table 3. Type of bacteria and concentrations of the alcoholic extract of ginger used, and inhibitory effectiveness

Bacteria	0.1mg /ml	0.2 mg /ml	0.3mg /ml
Enterobacter	Resistant	3 cm (Sensitive)	6 cm (Sensitive)

Ginger was extensively utilized for several applications, including as a cosmetic product, nutritious meals, condiment, as well as medicinal agent. Results showed that ginger extracts possess potential antibacterial activity against Enterobacter, as shown in Tables 1&2. The different secondary metabolites present in the extracts of ginger include simple phenolics, flavonoids, glycosides, alkaloids, saponins, tannins, reducing sugar, and carbohydrates. Tannins tend to dissolve in water. Terpenoid chemicals are lipophilic.

A triterpenoid is a terpenoid having antibacterial properties. Flavonoids are typically more soluble in aqueous solutions.

Glycosides are substances composed of sugar and non-sugar moieties. Saponins typically exist as glycosides, rendering them polar compounds. The antibacterial activity of plants is due to phenolic compounds, flavonoids, saponins, tannins, and essential oils [25]. Gingerols, the primary phenolic component in ginger, exhibit a range of bioactivities such as antibacterial effect [16]. The antimicrobial activity of ginger may stem from the significant amounts of phenolic compounds found in it [26].

Numerous papers demonstrate the inhibitory impact of ginger extract on different microorganisms. Prior research has shown moderate to strong antibacterial activities of ginger [27].

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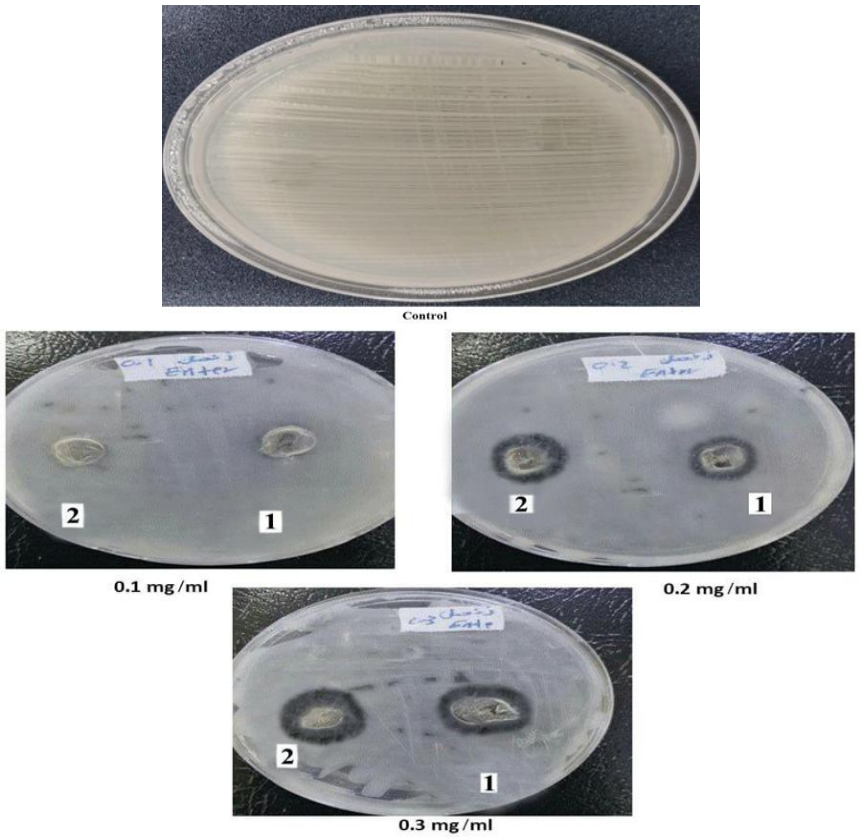

Figure 3. Inhibitory effectiveness of aqueous and alcoholic extracts of Enterobacter bacteria (0.1,0.2,0.3) mg/ml, (1 = Aqueous ginger extract, 2= Alcoholic ginger extract

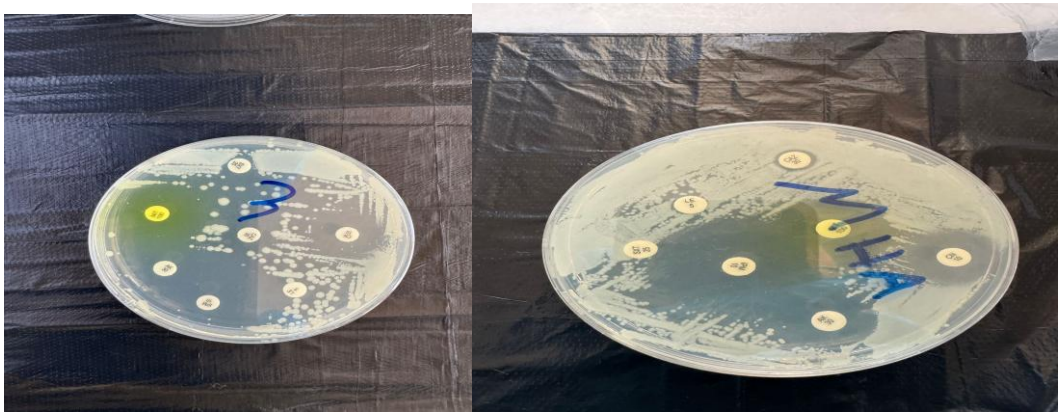

Figure 4. Bacterial isolates resistant to beta-lactam antibiotics

Gull demonstrated that methanolic and ethanolic extracts of ginger exhibited a substantial antibacterial impact on Escherichia coli isolates; however, the aqueous ginger extract was only marginally effective [28]. Ginger ethanol and methanol extracts exhibit superior efficacy against all examined bacterial strains compared to aqueous ginger extracts. *Shigella* and *E. coli* exhibited heightened susceptibility to the ginger extracts.

E. coli demonstrated the highest sensitivity to ethanol ginger extracts, but *Shigella* revealed maximal sensitivity for both ethanol and methanol ginger extracts. The findings about the antibacterial properties of ginger in the study are consistent with the majority of published literature on ginger's antibacterial efficacy [29].

The prior investigation documented the inhibitory effects of ginger extracts on eight drug-resistant pathogenic bacteria: *S.aureus, S.epidermidis, E.coli, K. pneumoniae, P. aeruginosa. B.subtilis,* and *S.typhi*[27].

The antibacterial efficacy of ginger was mostly ascribed to its secondary metabolism. Gingerol represents a bioactive secondary metabolite in ginger that's efficient against the *Enterobacteriaceae* family. Prior research on ginger has demonstrated that shogaols and gingerols are among its active constituents. The distinctive flavor and aroma of ginger result from a combination of gingerols, shogaols, and zingerone, which are volatile

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oils constituting 1–3% of the weight of fresh ginger [30,31]. The plant crude extracts are influenced by factors such as the chemical structure of the plant, solvents (polar or non-polar), the extract procedure, and sample size, thus affecting the biological activity of crude extracts [32].

The antibody method was used for all isolates under study for ten antibiotics from the thalaxam group, most of which are commonly used in the country for the treatment of different infections, to find out the type of response through determining the diameter of the inhibitory zone, and for the first results as reported [33]. The test results of antibiotics used in the study, where the resistance of *Enterobacter* isolates to (CAZ) was 90% and two sensitive isolates were 10% result correspond to the results of the researcher [34]. The percentage of *Enterobacter* isolates resistant to CAZ antibodies as consistent with the researcher's study If the percentage of *Enterobacter* isolates for resistance to CAZ in a study of the researcher[35], where the resistance ratio of *Enterobacter* isolates to CAZ drugs the resistance rate of *Enterobacter* isolates included in the study (CTX) was 25% cefotaxime, the percentage of moderate isolates sensitive to the antigen was 70%, and the percentage of isolates was 5%, this percentage corresponded with other previous study[36], where the resistance ratio of *Enterobacter* isolates to an antibody was 25.5%[36], these results differed from the researcher if the resistance ratio of *Enterobacter* isolates to the antibody was 91% SS.

The study showed that all isolates (100%) were resistant to the tested antibody (CL), aligning with the findings of the referenced researchers, and these results agreed with other previous studies [37]. Similarly, resistance to Cephalothin (CEP) was 100%, consistent with the findings of a previous study [37]. Regarding the anti-CEC antibody, 15% of isolates were resistant, while 30% were moderately sensitive, and 55% were sensitive to the antidote. These results differed from previous findings, as the resistance rate of Sidosunas isolates was reported to be 100%. For the anti-IPM test, the findings indicated that no resistance was observed, which agreed with other results [38]. The percentage of isolates resistant to the anti-IMP antibody also matched the results of that study, where resistance to Pseudomonas aeruginosa isolates reached a similar level. The results of the Meropenem (anti-MEM) test showed no resistance among internal isolates, with Enterobacter isolates showing 100% sensitivity to MEM. This outcome aligned with the findings of El-Far et al. [38].

The isolates included in her study did not show any resistance to the anti-meropenem, and the current study agreed with the study by EI-far *et al* [38]. The sensitivity of *Enterobacter* isolates to the anti-EME. The results of the Aztreonam isolation test were the emergence of one anti-EME resistant isolate 5% and 7 moderate isolations to the antidote 55% and 2 isolates showed their sensitivity to the antibody by 40%. Findings of this study diverged from those of the researcher's investigation, EI-far *et a*l [38]. The percentage of resistance of *Enterobacter* isolates to the anti-Aztreonam was 18.5% and the percentage of antidote isolates in a study. The resistance of *Enterobacter* isolates to Ticarcillin antibody (TI) was 25%, the result of this study corresponded with the study by EI-far *et a*l., as the percentage of resistance of *Enterobacter* isolates to the antibody included in the current study was medium sensitivity to the antibiotic. 30% of the isolates were sensitive to anti-TI. The percentage of isolates included in the study for the anti-PI Piperacillin was 20%, and 60% of the isolates of the current study were moderately sensitive to the antibiotic, and (20%) of the isolates of the current study showed their sensitivity to the anti-piperacillin this percentage corresponded to the study of the researcher Glen *et a*l., as the percentage of Simkunas isolates resistant to the antibody [39], as the resistance rate of this antibody reached 24% In the study, we observe a high percentage of resistance to *Enterobacter* isolates against anti-cephaloprons and penicillins such as ceftazidime, cephalothiin, cefaclor, cephalexin, piperacillin, and ticarcillin due to the ability of *Enterobacter* to produce beta-actamase enzymes as broad-spectrum ESBs.

This works on the analysis of penicillins and cephalosporins in particular, whose genes are carried either on chromosomes or on plasmids in many types of germs, which leads to multiple resistance to different antibiotics in addition to modifying the percentage of penicillin-binding proteins PBPs, which are the main target of beta-lactam. The reason for the resistance of *Enterobacter* to many generations of the cephalocyreins group is due to the germ's production of cephalosporin enzymes, which are one of the most important enzymes B_lactamase that encode genes carried on the comosome, in addition to other types of enzymes, including CARB-4, CARB-3, PSE-4, PSE.1. Which was found in the spores of *Enterobacter*, which is responsible for resistance to Pencillins, anticarbapenams, A Ztreoonam and first, second and third generation antidotes of cephalosporins, and the widespread and random use of these antibiotics by patients (in several cases) may lead to the emergence of resistance due to their availability and ease of use (oral) and cheap price, as the high rates of resistance to germs in general to antibiotics are a man-made problem and are global in spread, but they It is clearly manifested in the developing world compared to developed countries[40]. Due to the high sensitivity of the isolates involved in the study to antibodies of the Cariaein group, such as Imipenem, Meropenem may be due to their lack of production of the mineral beta-lactamase enzymes MBLs, if the phenotypic detection of MBLs enzymes shows that none of the isolates produce MBLs are produced. Betala enzymes as metallic amase, analyze antibodies of this group [40].

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Conclusion

Ginger extracts are more effective as supplements to support treatment rather than as substitutes for antibiotics, particularly in cases involving severe or resistant infections. Both aqueous and ethanolic ginger extracts contain compounds that act as strong antibacterial agents against *Enterobacter*. Nevertheless, additional research is required to evaluate the safety of these extracts, especially regarding their antibacterial efficacy, and to identify specific components responsible for these effects. It is essential to investigate the antibacterial properties of ginger extracts against other bacterial species that are resistant to commonly used antibiotics, as well as against fungal infections.

Conflict of interest. Nil

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