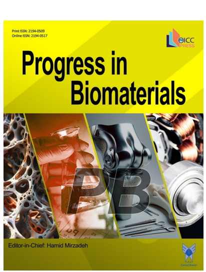

Volume 14, Issue 1, 142501 (1-16)

Progress in Biomaterials (PIBM)

https://doi.org/10.57647/pibm-2025-17530

Shogaol from *Zingiber officinale*: Antimicrobial, Anti-Virulence, and Therapeutic Potential against Foodborne Pathogens and Beyond

Mohamed A. Fareid[1,*], Gamal M. El-Sherbiny[2], Nancy M. Elafandy[1], Nagat E. Eltoum[3], Mohamed S. Othman[4], Mohamed H. Sharaf[2], Amr M. Shehabeldine[2], Eman E. Farghal[5], Fatma A. Hamada[6]

[1] *Clinical Laboratory Science Department, Applied Medical Science College, University of Ha'il, Saudi Arabia.*
[2] *Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Egypt.*
[3] *Clinical Nutrition Department, Applied Medical Science College, University of Ha'il, Saudi Arabia.*
[4] *Biochemistry Department, College of Medicine, University of Ha'il, Saudi Arabia.*
[5] *Clinical and Chemical Pathology Department, Faculty of Medicine, Tanta University, Egypt.*
[6] *First Year of Health and Medical Colleges, Basic Sciences Department, University of Ha'il, Saudi Arabia.*

[*] **Corresponding author:** m.alekhtaby@uoh.edu.sa

Original Research

Received:
3 January 2025
Revised:
14 March 2025
Accepted:
30 March 2025
Published in issue:
31 March 2025

© 2025 The Author(s). Published by the OICC Press under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Abstract:

Foodborne pathogens pose a considerable global challenge, acting as reservoirs of pathogens and contributing to the development of antimicrobial resistance. Herein, this study aims to extract shogaol from *Zingiber officinale* and use them in fighting foodborne pathogens and their virulence factors as well as potential medicinal benefits. The extracted shogaol have demonstrated significant antimicrobial action against foodborne bacterial species, with MIC ranging from 62.5 to 250 μg/mL and a reduction in biofilm formation of 66.33 $\pm$ 0.68%, as well as a significant decrease in the *fnbA*, *icaA*, and *can* genes expression levels. Furthermore, shogaol showed antioxidant activity through DPPH and ABTS assays with IC_{50} values of 174.32 and 185.89 μg/mL, and anti-hemolytic activity of 60.2 $\pm$ 0.261% to 78.5 $\pm$ 0.31% at concentration 4 and 50 μg/ml, respectively. Furthermore, shogaol revealed remarkable inhibitory action on α-glucosidase with (72.0%) and α-amylase (75.0%) and anticancer activity against two cancer cell lines, MCF7 ATCC-HPT-22 and HCT116 ATCC-CCL-247, with IC_{50} values of 98.41 $\pm$ 0.9 and 95.48 $\pm$ 0.6 μg/mL, respectively. GC–MS analysis demonstrated that the major active compound in *Zingiber officinale* extract is shogaol. Our findings highlight shogaol as a promising alternative approach to combat foodborne bacterial pathogens and their virulence factor.

Keywords: Shogaol; Antibacterial; Anti-virulence factors; Medicinal benefits

Cite this article: Fareid, M. A., El-Sherbiny, G. M., Elafandy, N. M., Eltoum, N. E., Othman, M. S., Sharaf, M. H., Shehabeldine, A. M., Farghal, E. E., Hamada, F. A. Shogaol from *Zingiber officinale*: Antimicrobial, Anti-Virulence, and Therapeutic Potential against Foodborne Pathogens and Beyond. *Progress in Biomaterials* **14**(1), Article 01 (2025).

1. Introduction

The escalating challenge of antibiotic-resistant bacteria, caused by the presence of biofilms and the development of resistance mechanisms, accentuates the urgent requirement for the formulation of novel and non-traditional pharmaceuticals. Historically, plant-based natural medicines have played a significant role in the fields of pharmacology, food, and cosmetics, with comprehensive records available from numerous countries. Ancient civilizations employed these substances not only as sources of nutrition but also for their medicinal properties [1, 2]. The bioactive compounds, also known as phytonutrients, generated by plants arise from their secondary metabolic processes [3, 4]. Some of these compounds demonstrate antimicrobial characteris-

tics against both pathogenic and spoilage microorganisms, resulting in a growing use in the food industry [5, 6].

Zingiber officinale (*Z. officinale*), a rhizome from a monocotyledonous annual plant, is indigenous to Africa and Asia and is widely utilized as a dietary supplement and food ingredient, as well as in traditional medicine across various cultures [6, 7]. Primarily, *Z. officinale* is rich with essential oils, alkaloids, phenolic compounds, flavonoids, glycosides, saponins, tannins, steroids, terpenoids, carbohydrates, and proteins and employed to enhance the flavours of traditional dishes and beverages. Nevertheless, owing to its numerous health benefits, its antimicrobial efficacy has been the subject of various studies. For instance, *Z. officinale* has shown promising inhibitory effects against pathogenic bacteria and fungi [8, 9, 10]. The predominant compound was found to be shogaol; 6-shogaol sees a marked increase in concentration during *Z. officinale* processing, primarily attributed to the heat-induced transformation. of 6-gingerol. Various studies have shown that 6-shogaol possesses notable pharmacological and biological effects, including antioxidant, anti-inflammatory, and anticancer activities. The anticancer mechanism of 6-shogaol encompasses the enhancement of paraptosis and apoptosis, an elevation in reactive oxygen species production, the initiation of autophagy, and inhibition of AKT/mTOR signaling [11]. Furthermore, research has indicated that shogaol possesses a range of beneficial properties, including analgesic [12], antipyretic [13], antiviral [14], antidiabetic [15], anti-inflammatory [16], antihelmintic [17], antitumor [18], antibacterial, antibiofilm, and antioxidant effects, making it a recommended remedy for various gastrointestinal and respiratory ailments [16]. Additionally, extensive research has been conducted to separate and identify shogaol, aiming to clarify the mechanisms underlying their antimicrobial efficacy toward both pathogenic and food spoilage microbes. A previous study reveals that shogaol has a wide antimicrobial spectrum against various microorganisms, positioning it as a promising alternative to synthetic antibacterial. Nevertheless, its utilization in food products remains underexplored, presenting a significant opportunity for future research [19]. In this regard, research indicates that 6-shogaol exhibits superior anticancer, anti-inflammatory and antioxidant properties compared to 6-gingerol. This advantage is likely due to the presence of an α, β-unsaturated carbonyl group (Michael receptor) in the chemical structure of 6-shogaol. It has been utilized in the treatment of various illnesses, involving cancer. The antitumor effectiveness of 6-shogaol has been validated across numerous cancer models, including those for breast, cervical, colon, liver, kidney, oral, and prostate cancers [19, 20, 21, 22].

Although several studies have been carried out on shogaol, the knowledge concerning its uses in the food industry remains scattered. Furthermore, these studies have not explored the potential applications of shogaol in the creation of functional foods, nor have they considered its potential in food packaging and edible coatings aimed at combating foodborne bacterial pathogens resistant to antibiotics and their virulence factors. This research offers a thorough investigation of extraction and the biological benefits of shogaol, which supports its use in functional foods and various roles within the food industry. Ultimately, it is the safe application of shogaol in functional foods, natural preservatives, and innovative food packaging. This innovative approach not only enhances food safety but also promotes health benefits, making it an asset in the ongoing quest for sustainable food solutions. Thus, the objective of this study was to extract shogaol from *Z. officinale* and evaluate its antibacterial and anti-virulence activities against foodborne pathogens as well as to explore its potential applications in food functions, particularly underscoring its antidiabetic, antioxidant, and anticancer properties.

2. Materials and methods

2.1 Chemicals and reagents

Muller Hinton broth, Mueller Hinton agar, and gentamicin, along with antibiofilm agents such as crystal violet, glacial acetic acid, diethyl ether, and n-hexane, were procured from Himedia in Mumbai, India. Each of these reagents was of analytical grade. Furthermore, the antioxidant substances, which include diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS)), potassium persulfate, and ascorbic acid, as well as anti-inflammatory compounds like sodium diclofenac and dimethyl sulfoxide (DMSO), and antidiabetic chemicals such as pig pancreatic α-amylase enzyme, dinitrosalicylic acid, α-glucosidase, and p-nitrophenyl-α-D-glucopyranoside, were obtained from Sigma-Aldrich based in St. Louis, MO, USA.

2.2 Plant material

Rhizomes of *Z. officinale* were purchased from a local market, Ha'il, Saudi Arabi. After thoroughly cleaning the rhizomes with running tap water to get rid of any impurities, they were rinsed with distilled water. After that, they were laid out on fresh paper towels and allowed to air dry for three days at room temperature (25 ± 2 °C). After drying, a high-speed electrical blender was used for five minutes to grind the rhizomes until a finely ground powder. To attain a consistent particle size, the powdered material was next passed through a 40-mesh screen. A Soxhlet apparatus (Model No. 3840, Borosil Glass Works Ltd., Mumbai, India) was used to extract 100 g of powdered *Z. officinale* rhizomes from a thimble containing 1 L of methanol over the course of 8 hours at 60 °C. Following passing the extract through Whatman No. 1 filter paper, the solvent was extracted using a rotating vacuum at lower pressure. On a 20×20 cm silica gel plate, the oily liquid extract was used in a stripe pattern. A 7:3 ratio of diethyl ether to n-hexane was used as the developing solvent. After the development procedure was finished, the plate was taken out 16 cm from the origin and analyzed using a UV lamp with the wavelength set to 254 nm. Using UV light, a blue band with an RF value of 0.15422 was seen; this was removed by scraping off, desorbed with methanol, and dried off. The conversion of 6-gingerol to 6-shogaol can occur in the presence of a strong acid or base, resulting in the dehydration of 6-gingerol through either protonation by a strong acid or deprotonation by a strong base. Therefore, the residue was

dissolved in a 1.5 N sulfuric acid diluted solution, refluxed for 12 hours, and then neutralized with a sodium hydroxide diluted solution. A solution of n-hexane and diethyl ether (4:6) was used twice to extract the mixture of shogaols and gingerols. It was then rinsed with distilled water, dried with anhydrous sodium sulfate, and concentrated to a small volume. Re-developing on a 20 × 20 cm TLC plate and shogaol reference (PubChem, Egypt) allowed for the isolation of the strong shogaol, which had an RF range of 0.40 to 0.45 [23, 24, 25].

2.3 Antibacterial activity of shogaol

To evaluate the antibacterial activity, the following bacterial strains were employed: *E. coli* (ATCC-8739) (E18) and *K. pneumoniae* (ATCC-9633) (K55) and foodborne bacterial species *E. coli* (E4), *K. pneumoniae* (K58), *P. aeruginosa* (A75), *P. aeruginosa* (A266), *B. cereus* (B9), and *S. aureus* (S70). The agar well technique was used to evaluate the antibacterial efficacy of shogaol using the aforementioned strains, which had been obtained from the bacteriology lab at the Faculty of Science, Al-Azhar University, Cairo, Egypt. The bacteria were cultured in Mueller Hinton Broth (MHB) and incubated for 24 hours at 37 °C. Mueller Hinton Agar (MHA) was then evenly coated with a 0.1 mL bacterial suspension with a McFarland turbidity of 0.5. A 50 µL of shogaols and a gentamicin disc (10 µg/mL) as a control were loaded on an agar well and agar surface and incubated at 4 °C for 2 hours to prime shogaols and a gentamicin diffusion. Following the plates being incubated for 24 hours at 37 °C, the zones of inhibition around the well discs were measured in millimeters (mm). The investigation was carried out in triplicate [26, 27].

2.4 Determination of the MIC values of shogaol

The MIC of shogaol toward foodborne bacterial species and standard strains was determined utilizing the microdilution broth assay in a 96-well microplate. Muller-Hinton broth (MHB) was added to every microplate well, and then 100 µL of a bacterial cell suspension that had been quantified to a concentration of 106 CFU/mL was added. While the amounts of shogaol ranged from 0.0 to 1000.0 µg/mL. For twenty-four hours, the microplates were incubated at 37 °C. The optical density (OD) at 630 nm was measured after the incubation. The lowest concentration that effectively prevented observable bacterial growth was known as the minimum inhibitory concentration (MIC) for shogaol [28, 29].

2.5 Antibiofilm properties of shogaol

The microplate technique was employed to quantitatively assess the capacity of shogaol to inhibit biofilm formation. A 0.5 McFarland turbidity of bacterial strains was prepared from cultures 24 hours old. Inoculate the well with 100 µL of the prepared diluted bacterial suspension. Fifty microliters of shogaol were introduced into the wells at three concentrations (1/8, 1/4, and 1/2 MIC). The experimental design included wells with shogaol and growth medium and growth medium with inoculum as a control. The wells underwent multiple washes with phosphate-buffered saline (PBS) to ensure proper cleaning. After a fixation period of 15 minutes in methanol, the plate was permitted to dry naturally. The staining process involved the application of 100 µL of a 1% crystal violet solution in water, with each well resting at room temperature for a duration of 30 minutes. Following this, a 100 µL solution of glacial acetic acid (GAA) at 33% concentration was utilized to rinse the stain, which was subsequently dried. The plate underwent three washes with distilled water. The optical density (OD) of each well was then assessed at 630 nm using an ELISA reader. The calculation of biofilm inhibition percentage was performed using the equation:
% inhibition = [OD negative control − OD medium control) − (OD test − OD shogaol control)]/(OD negative control − OD medium control) × 100 [9]. The investigation was carried out in triplicate, and the results were reported as mean ± standard deviation.

2.6 Effects of shogaol on expression of *cna*, *fnbA*, and *icaA* genes

Employing isolates of bacteria as a control that were not exposed to shogaol, the half MIC of shogaol was calculated and treated with a bacterial suspension at a 0.5 MacFarland. For twenty-four hours, the cultures were incubated at 37 °C. Using the primer sequences listed in Table 1, the expression levels of the genes (*cna*, *fnbA*, and *icaA*) were evaluated by qRT-PCR. The AMV reverse transcriptase enzyme (Roche, Basel, Switzerland) was used in conjunction with transcriptase to accomplish cDNA synthesis at a concentration of 25 units/L. The RNA extracted in the preceding stage was heated to 65 °C for three minutes in order to prevent the development of secondary structures. Utilizing 2 µL of random primer and two AMV reverse transcriptase enzymes, reverse transcription (RT) was carried out for 60 minutes at 42 °C. The AMV enzyme was then incubated and inactivated for five minutes at 99 °C. The $2^{-\Delta\Delta CT}$ method (sample CT minus control CT), where CT stands for the threshold cycle, was used to quantify gene expression [30, 31, 32].

2.7 Antioxidant efficacy of shogaol

The antioxidant properties of shogaol were achieved through two methods as follows.

2.7.1 DPPH technique

The DPPH technique, as described in the work by Shehata et al. [33], was used to evaluate the radical scavenging activity of shogaol. Several concentrations of shogaol were examined, including 1000, 500, 250, 125, 62.5, 31.25, 15.62, and 7.81 µg/mL. After fully combining 5 mL, a 0.1 mmol/L ethanol solution of DPPH was added. Ascorbic acid was used in the laboratory as a standard control. At 27 °C, the mixture was let to stand for 20 minutes. At 517 nm, the absorbance was then measured. The ascorbic acid and shogaol IC_{50} values, which indicate the concentration required to produce a 50% reduction in the initial DPPH concentration, were calculated. The specified equation was used to assess shogaol antioxidant capability. The DPPH scavenging

Table 1. Primers are employed to detect the of *cna*, *fnbA*, and *icaA* genes.

Gene	Forward Primer (5′ to 3′)	Reverse Primer (5′ to 3′)
icaA (intercellular adhesion gene)	GAGGTAAAGCCAACGCACTC	CCTGTAACCGCACCAAGTTT
fnbA (fibronectinbinding protein A)	AAATTGGGAGCAGCATCAGT	GCAGCTGAATTCCCATTTTC
cna (collagen binding protein)	AATAGAGGCGCCACGACCGT	GTGCCTTCCCAAACCTTTTGAGC
16S rRNA (housekeeping gene)	GGGACCCGCACAAGCGGTGG	GGGTTGCGCTCGTTGCGGGA

activity is calculated using the following formula:

$$\text{DPPH scavenging activity} = \frac{\text{control absorbance} - \text{different concentrations absorbance}}{\text{control absorbance}} \times 100$$

The antioxidant activity of the ascorbic acid and shogaol was measured as DPPH radical scavenging activity (%) and the IC_{50} DPPH values (shogaol concentration required to inhibit 50% of DPPH radicals) were calculated. This experiment was carried out in triplicate, with results conveyed as the mean value ± standard deviation.

2.7.2 ABTS technique

The antioxidant capabilities of shogaol were investigated utilizing the ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) cation radical decolorization method, which underwent slight modifications from the method presented by Shehata et al. [33]. In the study, ascorbic acid and shogaol concentrations were 1000, 500, 250, 125, 62.5, 31.25, 15.62, and 7.81 µg/mL. A 7 mmol/L ABTS solution and 2.4 mmol/L potassium persulfate were reacted in the dark for 12 to 16 hours at 25 °C to produce an ABTS cation radical. This solution was diluted in ethanol at a ratio of 1:89 (V/V) before the reaction started, and its absorbance was determined at 734 nm after it had been allowed to equilibrate at 30 °C. The antioxidant properties of the ascorbic acid and shogaol was calculated as ABTS scavenging activity (%) using the following equation

$$\text{ABTS scavenging activity} = \frac{\text{control absorbance} - \text{different concentrations absorbance}}{\text{control absorbance}} \times 100$$

The IC_{50} ABTS values (shogaol concentration required to inhibit 50% of ABTS radicals) were calculated.
Three duplicates of the investigation were carried out, and the mean values ± standard deviation were used to present the findings.

2.8 Anti-inflammatory properties of shogaol

The human red blood cell membrane stabilization assay was used to evaluate shogaol anti-inflammatory qualities. A young volunteer who had abstained from non-steroidal anti-inflammatory drug (NSAID) used for a few days prior to the experiment's start provided blood samples. An equivalent volume of Alsever solution, which is made up of 2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl, was mixed with the blood. After that, this mixture was centrifuged at 3,000 rpm. A 10% suspension was made after the precipitated cells were rinsed with saline. In dimethyl sulfoxide (DMSO) solutions, different quantities of sodium diclofenac and shogaol were prepared at 4, 8, 16, 32, and 50 µg/mL. One milliliter of phosphate buffer, two milliliters of hyposaline, and half a milliliter of human red blood cell (HRBC) suspension were mixed for every concentration. This was followed by centrifugation of the mixture for 20 minutes at 3,000 rpm and incubation at 37 °C for 30 minutes. Using a spectrophotometer set to 560 nm, the hemoglobin levels in the supernatant were measured in order to quantify HRBC hemolysis inhibition [34]. The percentage of hemolysis inhibition is calculated using the following formula: percentage of inhibition of hemolysis = (control absorbance − absorbance of sample)/(control absorbance) × 100.

2.9 Antidiabetic activity of shogaol

The anti-diabetic properties of shogaol were achieved through two methods as follows.

2.9.1 α-Amylase inhibition assay

To determine the anti-diabetic properties of shogaol, specimens containing 10 − 100 µg/mL of the compound were incubated for 10 minutes at 25 °C in 500 µL of 0.02 mol/L sodium phosphate buffer (pH 6.9, with 0.006 mol/L NaCl). The concentration of pig pancreatic α-amylase in each sample was 0.5 mg/mL. Acarbose was utilized as positive control in this study. After the incubation period, 500 µL of a 1% starch solution in the same buffer was added to the reaction mixture. 1.0 mL of dinitrosalicylic acid (DNS) was introduced to the mixture after it had been incubated for an additional 10 minutes at 25 °C. Following heating in boiling water for five minutes, the reaction was stopped and permitted to cool to room temperature. After diluting the reaction mixture with 10 milliliters of distilled water, the optical density (OD) was measured at 450 nanometers. To be used as a control, the combination containing the enzyme and the remaining reagents was kept apart from the specimen being tested sample. Using the specified equations, the α-amylase inhibition activity was assessed and displayed as a percentage of inhibition [35]. The formula for calculating percentage inhibition is: % inhibition = [Absorbance control − (Absorbance sample − Absorbance blank)/Absorbance control] × 100.

2.9.2 α-Glucosidase inhibition assay

Various doses of shogaol, ranging from 10 to 100 μl, were mixed with 100 µL of α-glucosidase (0.5 mg/mL) in a 0.1 mol/L phosphate buffer solution (pH 6.9) and incubated for

10 minutes at 25 °C to evaluate its enzyme inhibitory capacity. As a positive control, acarbose was used. Next came the introduction of a 50 µL aliquot of a 0.1 mol/L phosphate buffer (pH 6.9) that included 5 mmol/L p-nitrophenyl-α-D-glucopyranoside. A spectrophotometer was then used to record the absorbance at 405 nm after the reaction solutions had been incubated for five more minutes at 25 °C [36]. The test specimen was kept apart from the enzyme and other reagent mixture for control. The inhibition percentage of α-glucosidase was calculated using the following formula to describe the data regarding inhibitory activity: Inhibition % = [(Absorbance of sample − Absorbance

2.10 In vitro anticancer activity of shogaol

The anticancer potential of shogaol was evaluated using the MTT technique and two cancer cell lines: the colorectal carcinoma HCT116 ATCC-CCL-247 and the human breast carcinoma cell line MCF-7 ATCC-HPT-22. According to El-Sherbiny et al. [37], this technique is renowned for its exceptional precision and effective colorimetric analysis. The MTT test, which gauges metabolic activity, was used to assess cell survival and proliferative capacity during the triplicate trials. The cells were cultured for 24 hours after the culture media was replaced with different amounts of active metabolites (ranging from 0.0 to 1000 µg/mL). After this incubation, the cells were rinsed with cold PBS or fresh media, and then they were incubated with a 0.5 mg/mL MTT solution for two to five hours. Each received 200 µL of DMSO following the removal of the MTT solution. A microplate reader was used to measure each treatment's optical density (OD) at 570 nm, and certain formulas were used to calculate the proportions of survival of cells and dying cells.

$$\%\text{Cell viability} = \frac{\text{Treat cells}}{\text{Control cells}} \times 100$$

$$\%\text{Cell death} = \frac{\text{Control OD-Sample OD}}{\text{Control OD}} \times 100$$

2.11 Characterization of the *Z. officinale* extract and shogaol by GC-MS

The GC-MS spectroscopy technique outlined by Alghazzaly et al. [38] was employed to identify and analyze the methanolic extract of *Z. officinale* and purified shogaol, with a few minor adjustments. The purified shogaol and *Z. officinale* extract vwere dissolved in methanol of spectroscopy-grade quality. A Thermo Scientific Trace GC1310-ISQ mass spectrometer (Austin, Texas, USA), equipped with a direct capillary column measuring 25 mm in length, 0.25 µm in thickness, and 30 mm in internal diameter, was utilized to perform the GC-MS analysis. A 1 µL sample was inoculated at a temperature of 250 °C, utilizing a 1:30 ratio of helium to sample as the carrier gas. Following five minutes at 50 °C, the oven gradually increased to 230 °C at a rate of five °C per minute for the subsequent two minutes. Operating in electron ionization (EI) mode at temperatures of 200 °C and 70 eV, the mass spectrometer displayed a scanning range from 40 to 1000 m/z. A comparison was conducted between the spectra of the resulting molecules and those of chemicals present in the NIST 11 and WILEY 09 libraries (Wiley, New York, NY, USA).

2.12 Statistical analysis

The data are presented as mean ± standard deviation, and the procedures were carried out in triplicate. To compare the control and experimental groups, a two-way ANOVA was used. To find statistically significant variations (p-value < 0.05), statistical evaluations were carried out utilizing GraphPad Prism Software version 8.0 (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results and discussion

3.1 Antibacterial activity and MIC of shogaol

One hundred grams of *Z. officinale* powder were extracted using methanol, yielding 7.8 grams from the crude extract and recovering 1.9 grams from shogaol. The shogaol showed considerable antibacterial activity against a variety of standard bacterial strains as well as foodborne bacterial species. The antibacterial activity assay revealed that shogaol exhibits significant inhibition zones ranging from 15.83 ± 0.44 to 23 ± 0.57 mm compared with gentamicin as a positive control (0.0 to 20 ± 0.57 mm), as illustrated in Table 2 & figure 1 Numerous investigations have demonstrated the antibacterial properties of shogaol toward various bacterial species obtained from clinical specimens as well as standard strains [16, 39, 40]. In a study on shogaol's antibacterial qualities, Shareef et al. [41] used the technique of diffusion of agar to compare the drug's effectiveness against common antibiotics against *E. coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. Their findings indicated that shogaol exhibited a more potent antimicrobial effect than streptomycin, rifampicin, and cefotaxime. Food spoilage microorganisms present significant challenges for many developing nations. These microorganisms can adversely affect the taste, texture, and appearance of food, ultimately diminishing its nutritional value. Food that is contaminated becomes unsuitable for human consumption. To mitigate or eradicate pathogenic bacteria in food, various strategies must be implemented. These strategies not only enhance food quality and safety but also extend its shelf life [39]. Furthermore, the antimicrobial properties of shogaol have been evaluated against several pathogens, using an agar diffusion approach, such as *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. According to reports, shogaol successfully suppresses foodborne pathogens such *Enterococcus species*, *S. aureus*, and *E. coli* [42, 43, 44, 45, 46]. The antibacterial effects of ginger are mostly attributed to its abundance of volatile and aromatic chemicals. In addition to improving food's nutritional content, these aromatic qualities also help preserve it better than artificial or chemical preservatives. The composition of the substrate, processing techniques, and storage time all have an impact on shogaol's ability to inhibit microbial activity [40].

In this study, shogaol had MICs against tested bacterial strains between 62.5 and 250 μg/mL against a variety of standard bacterial strains and foodborne bacterial species,

Table 2. Antibacterial action of shogaol against different bacterial strains.

Bacterial species	**Mean of inhibition zone diameter mm (mean ± SD)**			
	DMSO	Shogaol	Gentamicin	*P*-value
E. coli (ATCC-8739) (E18)	0.0	15.83 ± 0.44	20 ± 0.57	0.0006
K. pneumoniae (ATCC-9633) (K55)	0.0	23 ± 0.57	18 ± 0.57	0.0004
E. coli (E4)	0.0	15.33 ± 0.33	17.5 ± 0.28	0.001
K. pneumonia (K58)	0.0	16.5 ± 0.28	0.0	<0.0001
P. aeruginosa (A75)	0.0	20.16 ± 0.44	0.0	<0.0001
P. aeruginosa (A266)	0.0	21.66 ± 0.33	0.0	<0.0001
B. cereus (B9)	0.0	22 ± 0.57	0.0	<0.0001
S. aureus (S70)	0.0	20 ± 0.57	17.83 ± 0.44	0.006

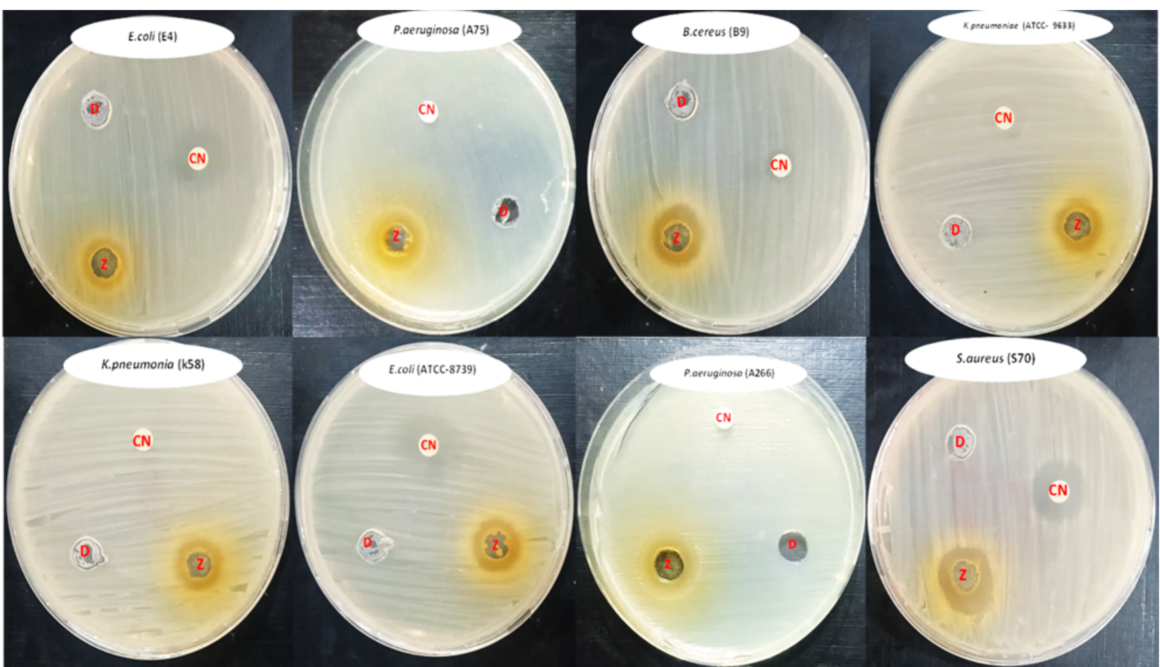

Figure 1. Antibacterial activity of shogaol against different bacterial strains, (Z) = shogaol, D = DMSO (Negative control), CN = gentamicin (positive control).

Table 3. MIC of shogaol against different bacterial strains.

Bacterial strains	Shogaol (μg/mL)
E. coli (ATCC-8739) (E18)	125
E. coli (E4)	125
K. pneumonia (K58)	250
S. aureus (S70)	250
K. pneumoniae (ATCC- 9633) (K55)	62.5
B. cereus (B9)	125
P. aeruginosa (A75)	250
P. aeruginosa (A266)	125

as shown in Table 3 and figure 2. The MICs of 4-shogaol that effectively inhibited the growth of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Serratia marcescens* were recorded at 0.4, 0.2, 0.4, 0.2, and 0.4 mg/mL, respectively. The antibacterial effects are linked to various mechanisms, with a primary focus on intending the bacterial cell membrane and disrupting its efflux capabilities, which are common modes of action for many antibiotics and biocides [47]. Koshak et al. [23], revealed that shogaol illustrated significant antimicrobial efficacy

against various bacterial species with an MIC of 75.6 µg/mL for *E. coli*, 67.0 µg/mL for *P. aeruginosa*, 70.2 µg/mL for *Proteus* sp., 68.5 µg/mL for *S. aureus*, and 4.5 µg/mL for *Bacillus* sp. However, it was noted that the shogaol demonstrated limited antimicrobial efficacy against *Enterobacter* sp. with MIC 185.6 µg/mL and *Klebsiella* sp. with MIC 185.6 µg/mL.

3.2 Antibiofilm activity of shogaol against different bacterial strains

The results concerning the invitro anti-biofilm activity of the shogaol bioactive compound against standard bacterial species and foodborne bacterial species demonstrated that shogaol significantly decreased biofilm formation in a concentration-dependent manner across all tested species. The most pronounced suppression was recorded for *S. aureus* (S70), with a reduction ranging from 66.33 $\pm$ 0.68% at 1/2 MIC to 29.92 $\pm$ 3.76% at 1/8 MIC. In contrast, the least reduction was observed in *E. coli* (E4), where the inhibition percentages varied from 24.42% at 1/2 MIC to 5.81% at 1/8 MIC as shown in figure 3. Shogaol has been shown in numerous studies to have the ability to stop the production of biofilms in a variety of bacterial strains that were taken from samples from hospitals [16, 48, 49, 50, 51]. When it comes to infections and antibiotic resistance, biofilm development is essential. By altering the membrane's integrity and inhibiting the production of biofilms, studies have shown that ginger can stop the growth of a *Pseudomonas aeruginosa* strain that is resistant to many drugs [52]. Furthermore, it has been demonstrated that shogaol treatments inhibit the formation of biofilms by lowering *Pseudomonas aeruginosa* PA14's levels of bis-(3-5)-cyclic dimeric guanosine monophosphate (c-di-GMP) [53]. Additionally, through downregulating virulence-associated genes, ginger's crude extract and methanolic fraction have been shown to inhibit *Streptococcus mutans* adhesion, glucan production, and biofilm formation. Consistent with in vitro results, a group of treated rats showed less caries formation linked to *Streptococcus mutans* [54]. Moreover, an in vitro study showed that 6-shogaol and gingerenone-A inhibited *S. aureus* by preventing the pathogen's 6-hydroxymethyl-7, 8-dihydropterin pyrophosphokinase from functioning [55].

3.3 Effects of shogaol on expression genes coded in biofilm formation

The findings demonstrate that bacterial strains exposed to shogaol at half the minimum inhibitory concentration for an overnight period showed a significant decrease the expres-

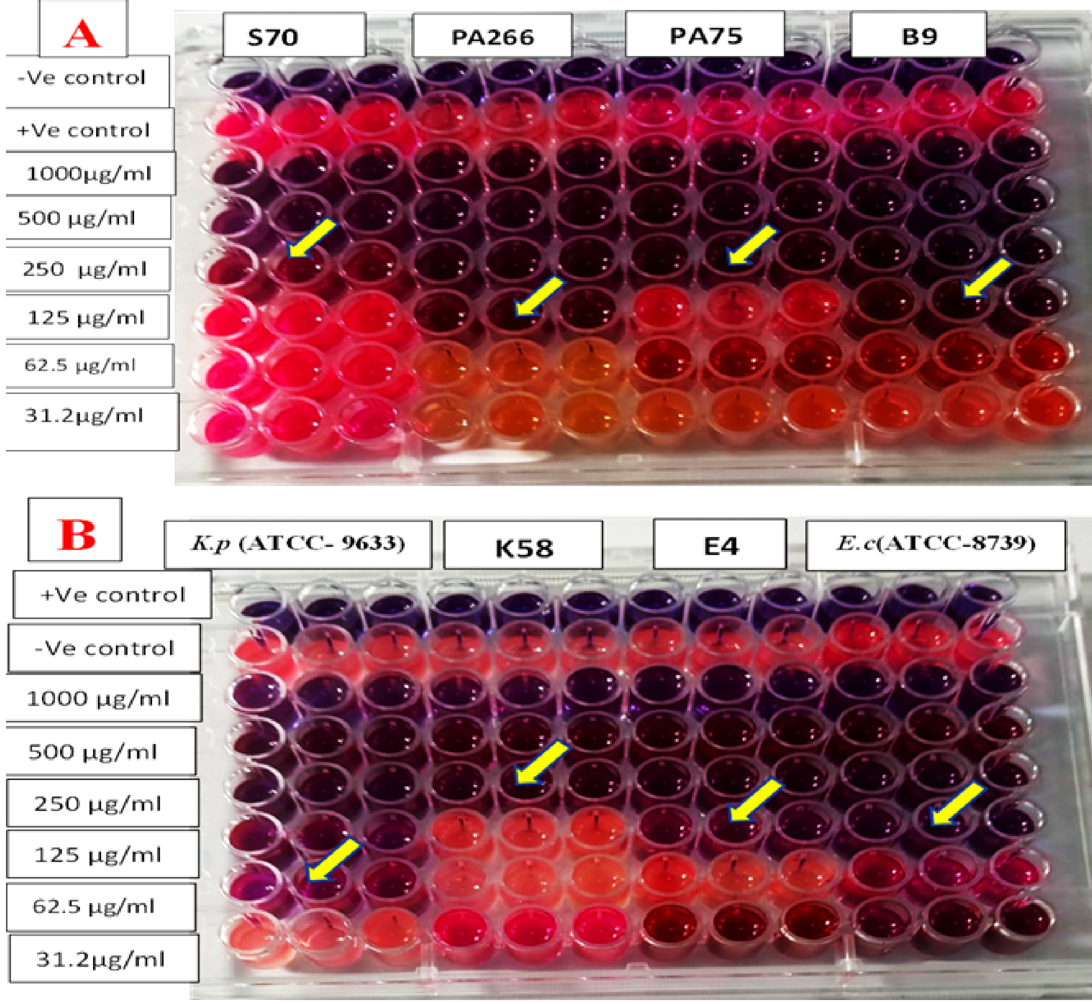

Figure 2. A and B: MIC of shogaol against a variety of standard bacterial species and foodborne bacterial species.

Figure 3. Antibiofilm activity of shogaol against different bacterial strains (**** P < 0.0001).

sion levels of the *fnbA*, *icaA*, and *cna* genes were found to be 2.0 to 2.3, 3.6 to 2.7, and 2.9 to 5.2, respectively (**** P < 0.0001), in comparison to the control isolates. This illustrated that shogaol plays a role in the downregulation of genes linked to biofilm formation, as depicted in figure 4. The results match what Lee et al. [56] found, showing that 6-gingerol, 8-gingerol, and 6-shogaol successfully stopped biofilms from forming. In particular, 6-shogaol at a concentration of 10 µg/mL greatly reduced the biofilm formation of *C. albicans* without affecting the growth of free-floating cells. Notably, 10-shogaol at a dosage of 10 µg/mL dramatically reduced *C. albicans* biofilm development without affecting planktonic cell proliferation. Shogaol's ability to disrupt bacterial quorum sensing (QS) systems was linked to its anti-virulence properties. This was shown by its interaction with QS receptors and the downregulation of QS-related genes. Crucially, 4-shogaol demonstrated a strong binding affinity to a number of QS targets in *P. aeruginosa*, according to in silico research [23].

3.4 Antioxidant activity of shogaol

An antioxidant is defined as any molecule capable of preventing, delaying, or significantly inhibiting the oxidation of an oxidizable substrate. The excessive generation of free radicals, particularly reactive oxygen species (ROS), is recognized as a major factor contributing to the onset and progression of numerous chronic diseases. Among natural antioxidants, flavonoids and phenolic compounds-secondary metabolites widely produced by plants-play a crucial role. These bioactive molecules protect biological systems from oxidative damage and, as a result, may provide substantial health benefits, including protection against cancer, cardiovascular diseases, atherosclerosis, diabetes, and various metabolic disorders [57]. In the present study, the antioxidant potential of shogaol, the major active constituent of *Zingiber officinale*, was evaluated using DPPH and ABTS radical scavenging assays. Shogaol exhibited IC_{50} values of 174.32 µg/mL and 185.89 μg/mL for DPPH and ABTS assays, respectively. In comparison, the standard

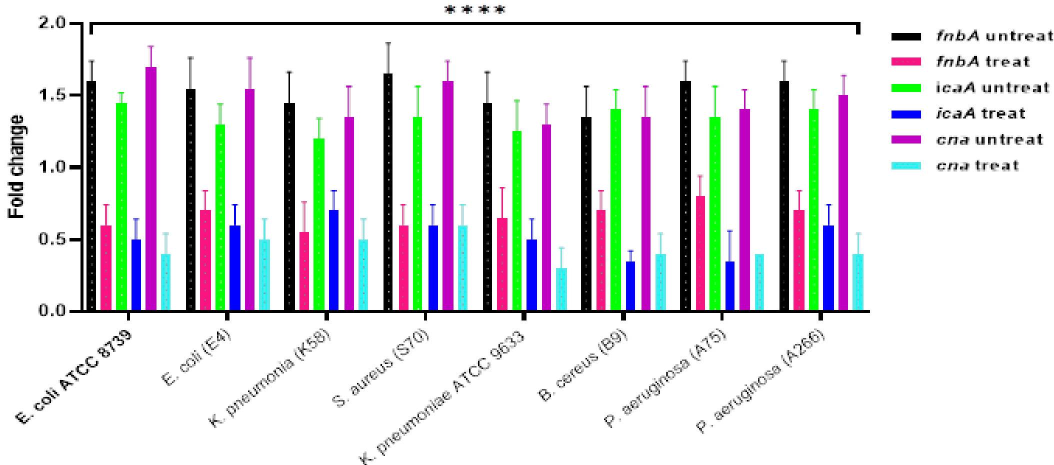

Figure 4. qRT-PCR expression of the biofilm-associated genes (*fnbA*, *icaA* and *cna*) in bacterial strains treated with shogaol, 1/2 MIC level and untreated (control) (*** P < 0.0001).

antioxidant ascorbic acid demonstrated markedly stronger activity, with IC_{50} values of 7.61 µg/mL and 7.50 µg/mL, respectively. These findings indicate that, although shogaol possesses antioxidant activity, its potency is considerably lower than that of ascorbic acid., as illustrated in figure 5A and 5B. In contrast with findings, several studies have demonstrated that shogaol possesses considerable antioxidant activity [16, 57, 58]. This may be attributed to the structure of shogaols, which are dehydration products of gingerols (formed during drying or heating), or the loss of a hydroxyl group reduces the hydrogen-donating ability, which is crucial for free radical scavenging [59, 60, 61, 62]. The IC_{50} value is inversely correlated with the free radical scavenging activity or antioxidant capacity of the sample. This means that a lower IC_{50} value indicates higher antioxidant activity, as less of the sample is required to effectively scavenge free radicals, and vice versa [29, 33]. Overall, ginger has been utilized for an extended period as a flavouring agent and is recognized for its antioxidant properties; it could help treat ailments like fever, dementia, indigestion, sore throat, and arthritis. Moreover, its anti-inflammatory effects may also support overall immune health, making it a valuable addition to various diets. Incorporating ginger into meals or teas can enhance both flavour and wellness benefits [39].

3.5 The anti-inflammatory action of shogaol

Shogaol bioactive molecules exhibited significant antihemolytic activity against human red blood cells in comparison to the positive control, sodium diclofenac, as illustrated in figure 6. The ability of shogaol to prevent hemolysis of human erythrocytes was recorded to be between 60.2 ± 0.261% and 78.5 ± 0.31% at concentration 4 and 50 µg/mL respectively, while sodium diclofenac demonstrated an inhibition range of 66.4 ± 0.141% to 83.1 ± 0.212%. at same concentration. A series of investigations has revealed that shogaol and its active ingredients have anti-inflammatory effects, which could provide protection against diseases linked to inflammation, such as colitis; These anti-inflammatory actions are predominantly connected to the signaling pathways of phosphatidylinositol-3-kinase (PI3K), protein kinase B (Akt), and the nuclear factor kappa light chain-enhancer of activated B cells (NF-κB) [16, 56, 63, 64]. Additionally, in human intestinal cell models, 6-shogaol showed protective

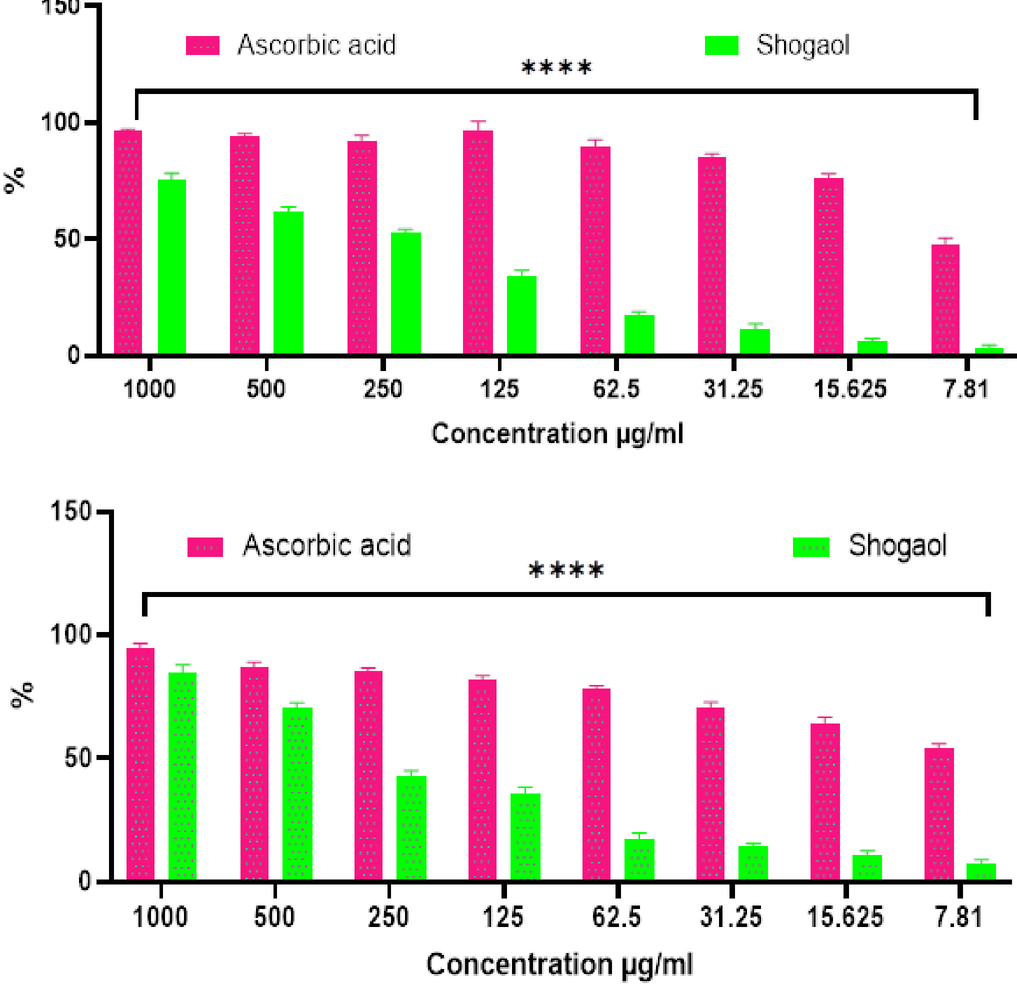

Figure 5. The antioxidant activity of shogaol (A) DPPH and (B) ABTS techniques (**** $P < 0.0001$).

qualities against intestinal barrier dysfunction brought on by tumor necrosis factor α (TNF-α). By blocking the signaling pathways linked to PI3K/Akt and NF-κB, it successfully prevented the overexpression of Claudin-2 and the disintegration of Claudin-1 [65]. Furthermore, 6-dehydroshogaol was more effective than 6-shogaol and 6-gingerol at reducing the synthesis of proinflammatory mediators in mouse macrophage RAW 264.7 cells, such as nitric oxide (NO) and prostaglandin E2 (PGE2) [66]. Likewise, it was discovered that zingerone and shogaol extract suppress NF-κB activation and lower IL-1β levels in mice's colons, consequently reducing colitis brought on by 2, 4, 6-trinitrobenzene sulfonic acid [67]. Additionally, ginger protected mice from enteritis brought on by anti-CD3 antibodies while also lowering TNF-α production and Akt and NF-κB activation [68].

3.6 Antidiabetic activity of shogaol

Diabetes mellitus is a serious metabolic disease that is typified by an inability to use insulin and/or resistance to its effects, which causes blood glucose levels to rise abnormally. Advanced glycation end products (AGEs) and protein glycation may be accelerated by prolonged hyperglycemia [69]. Shogaol bioactive molecules demonstrate the ability to inhibit key enzymes, specifically α-amylase and α-glucosidase, thereby proving to be effective in the control of diabetes. The shogaol exhibited a dose-dependent reduction in α-amylase activity, with inhibition levels ranging from 15% to 75% across various concentrations as shown in figure 7. In comparison, the conventional medication acarbose displayed a broader inhibition range of 17% to 84%. at equivalent doses. The suppressed effect of shogaol on α-amylase is due to its significant concentration of phenolic compounds. A variety of research studies have investigated the antidiabetic properties of ginger and its primary active components. These studies suggest that shogaol may play a crucial role in managing blood sugar levels by inhibiting carbohydrate digestion. As a result, incorporating ginger into one's diet could provide potential benefits for individuals seeking to improve their metabolic health [70]. Studies reveal that phenol-rich plant extracts (6-gingerol, 8-gingerol, and 10-gingerol) are superior to other compounds in their ability to inhibit α-amylase [57]. According to an in vitro study, 6-gingerol and 6-shogaol both successfully prevented the development of diabetes complications by ensnaring methylglyoxal (MGO), a precursor to advanced glycation end products (AGEs), and preventing the synthesis of AGEs [71]. Furthermore, 6-gingerol was shown to decrease insulin and plasma glucose levels in mice who were obese due to a high-fat diet. By activating Nrf2, 6-shogaol also decreased the levels of Nε-carboxymethyl-lysine (CML), a known indicator of AGEs. By encouraging AMPK phosphorylation, 6-paradol and 6-shogaol both improved glucose consumption in 3T3-L1 adipocytes and C2C12 myotubes. Also 6-paradol dramatically lowered blood glucose levels in a mouse model fed a high-fat diet [72]. Another study found that 6-shogaol increased glucagon-like peptide 1 (GLP-1) levels in type 2 diabetic mice, improving glucose-stimulated insulin secretion and glucose tolerance [73]. Additionally, 6-shogaol administration promoted glycogen storage in skeletal muscle by activating glycogen synthase 1 and upregulating the expression of glucose transporter type 4 (GLUT4) in the cell membrane. Additionally, in those with type 2 diabetes mellitus, ginger consumption has been demonstrated to reduce insulin, triglycerides (TG), total cholesterol (TC), glycated hemoglobin A (HbA1C), and fasting plasma glucose. [74]. Likewise ginger extract administration has been demonstrated to improve insulin sensitivity in rats with metabolic syndrome; this effect may be related to the enhancements in energy metabolism that 6-shogaol causes [75].

3.7 Anticancer activity of shogaol

According to projections from the International Agency for Research on Cancer (IARC), by 2050, it is anticipated that there will be 35 million new instances of cancer. Nevertheless, strategic investments in prevention, particularly those aimed at critical risk factors like smoking, obesity, and infections, have the potential to prevent millions of cancer diagnoses and preserve numerous lives worldwide [76]. Many

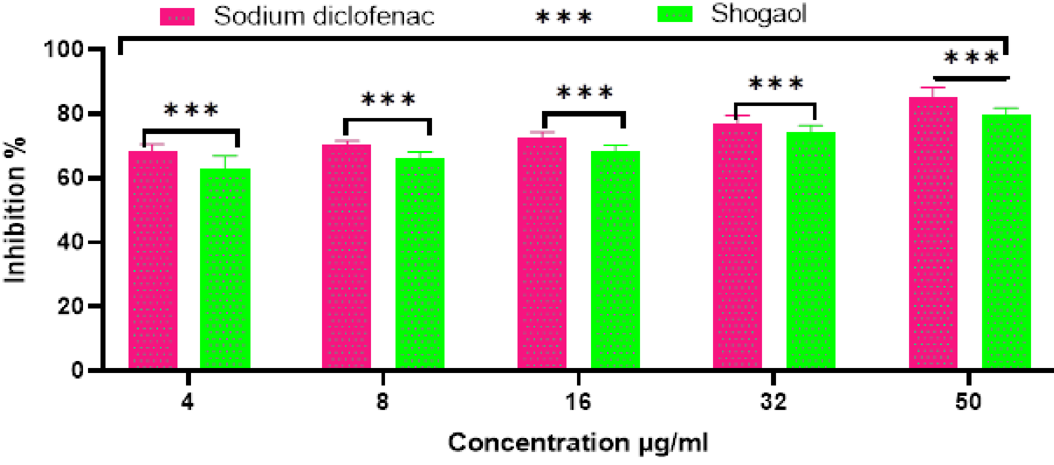

Figure 6. Anti-inflammatory activity of shogaol (*** P < 0. 001).

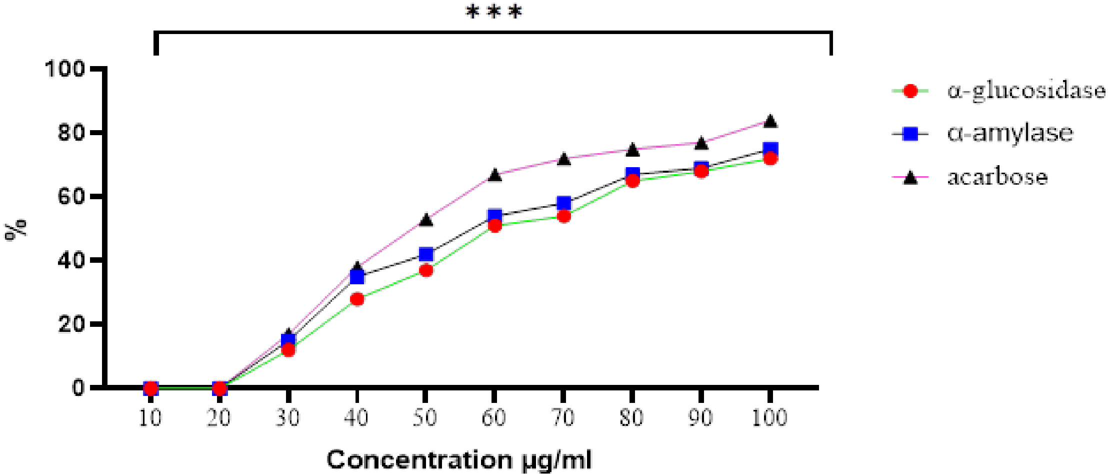

Figure 7. Antidiabetic activity of shogaol (*** P < 0. 001).

studies have indicated that products from nature, including fruits and medicinal plants, exhibit anticancer effects [57, 64]. In this study, shogaol exhibits significant anticancer action against two different types of cancer: the MCF7 ATCC-HPT-22 human breast carcinoma cell line and the HCT116 ATCC-CCL-247 colorectal carcinoma with IC_{50} 98.41 ± 0.9 and 95.48 ± 0.6, respectively. It is possible to identify and correlate morphological changes at the cell surface or inside the cytoskeleton with cell viability. The loss of proteins and intracellular ions along with altered permeability to sodium or potassium causes damage, which is typified by dramatic decreases in cell volume. In addition to cell shrinkage, nuclear condensation, and nucleus disintegration, notable indicators of injury include nuclear swelling, chromatin flocculation, and a decrease in nuclear basophilia, as shown in figure 8A and 8B. Shogaol has recently attracted a lot of attention due to its possible anticancer effects against a number of cancer types, such as prostate, colorectal, breast, and cervical cancers [57, 64, 77]. Numerous studies have demonstrated that ginger and its bioactive ingredients help prevent colorectal cancer-related carcinogenesis. A polyphenol-rich fraction of dried ginger powder was shown in an in-vitro investigation to efficiently inhibit the growth of gastric adenocarcinoma cells and colorectal cancer cells [78]. Furthermore, by suppressing the expression of genes linked to the Ras/extracellular signal-regulated kinase (ERK) and PI3K/Akt signaling pathways, including the v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), ERK, Akt, and B-cell lymphoma-extralarge (Bcl-xL), shogaol treatment caused apoptosis [79]. Additionally, it led to a rise in caspase 9 expression, which helped HT-29 colorectal cancer cells undergo apoptosis. Ginger extract administered in coated alginate beads increased the activities of succinate dehydrogenase and NADH dehydrogenase in rats with colon cancer caused by 1,2-dimethylhydrazine [80]. It has been discovered that gogaol has cytotoxic action against a variety of malignancies, such as those of the pancreas, liver, cervix, and breast. By lowering the protein levels of cyclin A and cyclin D1, 6-shogaol can stop the growth of HeLa human cervical cancer cells, causing cell cycle arrest during the G0/G1 phase, according to in vitro research. Furthermore, increased caspase production and suppression of the mammalian target of rapamycin (mTOR) signaling pathway promoted HeLa cell apoptosis [81].

3.8 GC-MS spectra of the *Z. officinale* extract and purified shogaol

Approaches used for the characterization or quantitative analysis of plant components in botanical extracts have involved gas chromatography-mass spectrometry (GC-MS) [82]. As illustrated in figure 9A and Table 4, the GC-MS analysis of the methanol extract from *Z. officinale* indicated that the most significant compound was 1-(4-Hydroxy-3-methoxyphenyl) dec-4-en-3-one (shogaol), which constituted 73.2% of the extract. The subsequent compounds included 1-(4-hydroxy-3-methoxyphenyl) tetradec-4-en-3-one at 5.73%, gingerol at 3.70%, and 1-(4-hydroxy-3-methoxyphenyl) dodec-4-en-3-one (dehydrogingerdione) at 2.61%. The purified shogaol exhibited a major peak at m/z 137 in the mass spectrum, consistent with its fragmentation pattern. It has a molecular formula of $C_{17}H_{24}O_3$ and a molecular weight of 276 g/mol. GC–MS analysis confirmed the purity of the final compound isolated from the methanolic extract of *Zingiber officinale*, as illustrated in figure 9B. The primary active constituents in ginger are gingerols, especially 6-shogaol, which stands out as the most significant [83]. Other variants, such as 4-, 8-, 10-, and 12-gingerols, are found in reduced concentrations. These gingerols primarily exhibit ginger's spiciness; however, they can be converted into shogaols when heated or dehydrated, resulting in the unique spicy-sweet scent that ginger is known for. It is important to recognize that fresh ginger does not have zingerone, although this compound can be formed from gingerols via hydrolysis when heated [84, 85]. The examination was conducted by Ersedo et al. [39] illustrated that the principal compounds in ginger, such as shogaol, paradol, and gingerols, have bioactive properties that can provide health benefits by enhancing metabolic and digestive systems during the cooking phase and after

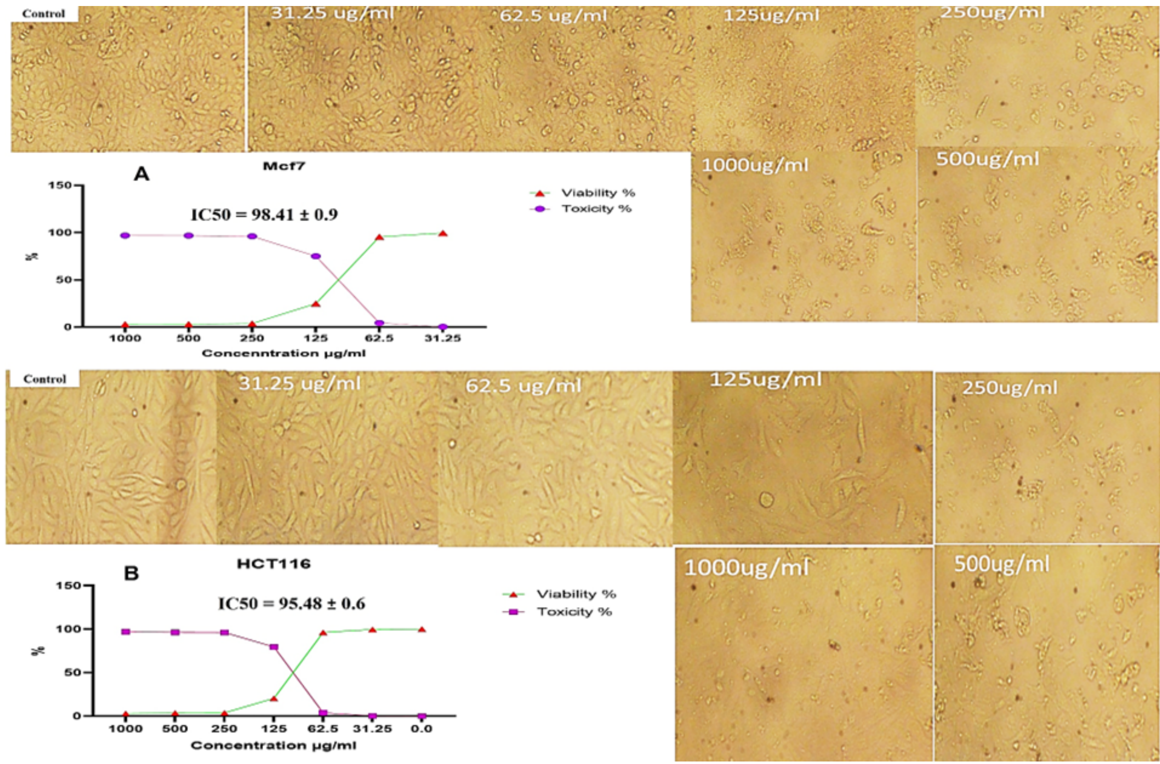

Figure 8. Anticancer activity of shogaol against two types of cancer cell lines (A) MCF7 and (B) HCT116.

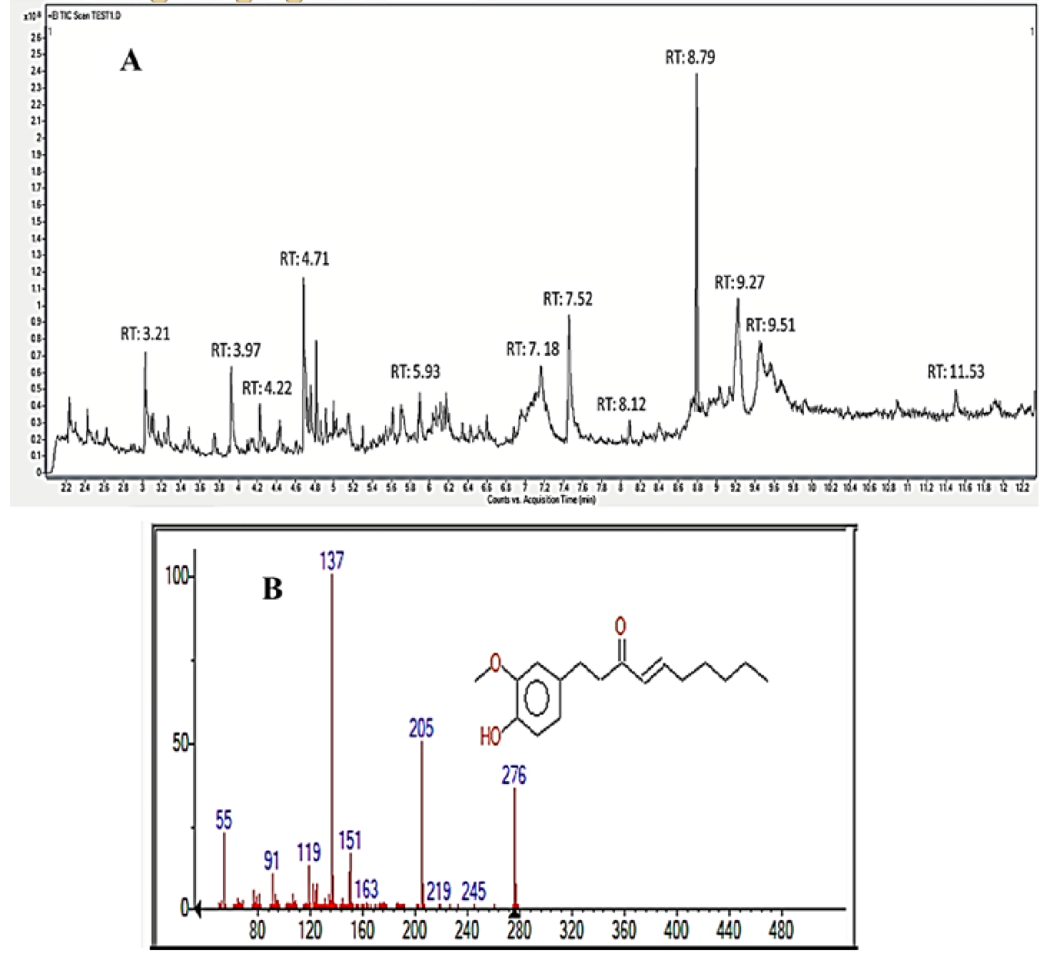

Figure 9. GC-MS (A) methanolic extract of *Z. officinale* and (B) purified shogaol.

ingestion. Furthermore, ginger is abundant in phenolic constituents, primarily consisting of gingerols, shogaols, and paradols. In fresh ginger, the predominant polyphenols are gingerols, which include 6-shogaol,8-gingerol, and 10-gingerol. Upon exposure to heat or extended storage, these gingerols may be converted into shogaols. In addition, shogaols can undergo hydrogenation to yield paradols. Ginger also encompasses a range of other phenolic ingredients, such as quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione [57, 86]. Sesquiterpene hydrocarbons, diarylheptanoids, and compounds related to gingerol constitute the primary components of ginger, accounting for $10-15\%$ of its composition. Additionally, these compounds can be categorized into five principal groups: gingerols, which are homologues of 1-(3-methoxy-4-hydroxyphenyl)-3-keto-5-hydroxyhexane and include a subgroup known as methyl gingerols; shogaols, which are formed through the dehydration of gingerols; paradols, which are β-ketone hydroxyl deoxygenation derivatives of gingerols; gingerdiones, which are β-ketone hydroxyl dehydrogenation derivatives of gingerols and encompass the subgroup 1-dehydro gingerdiones; and gingerdiols, which are products of the ketone reduction of gingerols [81].

4. Conclusions

The comprehensive in vitro investigation conducted in this study highlighted the significant food additive and therapeutic characteristics of shogaol. Shogaol exhibited significant antioxidants, anticancer, antidiabetic, anti-inflammatory, antibacterial, and antibiofilm activities, positioning it as a promising natural remedy for a range of health concerns. The antioxidant capabilities of shogaol, demonstrated through its ability to scavenge free radicals, suggest a protective effect against damage caused by oxidative stress. Furthermore, its capacity to inhibit key enzymes involved in glucose metabolism, such as α-amylase and α-glucosidase, indicates its potential as a natural treatment for diabetes. Also, shogaol exhibits considerable anti-inflammatory effects, as demonstrated by its ability to reduce hemolysis in human erythrocytes, which points to its potential in treating inflammatory disorders. In addition, shogaol showcases impressive antibacterial and antibiofilm capabilities, particularly against strains resistant to conventional drugs, highlighting its promise as a natural alternative to standard antibiotics. Phytochemical studies indicate that the *Z. officinale* extract contains a high level of bioactive compounds, such as shogaol and gingerol, with shogaol being the most abundant component identified via GC-MS analysis. Given its multifunctional therapeutic properties, shogaol presents significant potential, warranting further exploration of its pharmacological and medicinal benefits. Furter studies are needed to explore these properties and their implications for medicinal use.

Funding: This research has been funded by Scientific Research Deanship at University of Ha′il - Saudi Arabia through project number: RG-24 115.

Acknowledgments: The authors would like to thank the Scientific Research Deanship at University of Ha′il - Saudi Arabia.

Authors contributions

All authors contributed equally to the conception, design, execution, and writing of this work. All authors read and approved the final manuscript.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interests

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.

Table 4. Chemical profiling of the methanolic extract of *Z. officinale* by GC-MS spectrometry.

No	Compound	Molecular Formula	Contents %	Molecular Weight	Activity	Ref.
1	1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one (shogaol)	$C_{17}H_{24}O_3$	73.2	276	Anti-inflammatory, antibacterial, antiulcer, anticancer, antipyretic, antiviral, antidiabetic, anti-helmintic	[15]; [16]; [17]; [18]; [19]
2	1-(4-Hydroxy-3-methoxyphenyl)tetradec-4-en-3-one	$C_{10}H_{10}O_4$	5.73	194		
3	gingerol	$C_{17}H_{26}O_4$	3.70	294		
4	1-(4-Hydroxy-3-methoxyphenyl)dodec-4-en-3-one (Dehydrogingerdione)	$C_{19}H_{26}O_4$	2.61	318		
5	(E)-1-(4-Hydroxy-3-methoxyphenyl)hexadec-4-en-3-one [(E)-[12]-Shogaol]	$C_{23}H_{36}O_3$	2.51	360		
6	6-(3,5-Dimethyl-furan-2yl)-6-methyl-hept-3-one	$C_{14}H_{20}O_2$	1.72	220		
7	(E)-1-(4-Hydroxy-3-methoxyphenyl)dodec-4-en-3-one (8-Shogaol)	$C_{19}H_{28}O_3$	1.82	304		
8	3-(6-Hydroxy-3,7-dimethyl-oct-4-en-3-one	$C_{17}H_{24}O_3$	1.88	276		
9	1-(4-Hydroxy-3-methoxyphenyl)oct-4-en-3-one (4-Shogaol)	$C_{15}H_{20}O_3$	1.86	248		
10	5-Hydroxy-1-(4-Hydroxy-3-methoxyphenyl)oct-4-en-3-one	$C_{12}H_{14}O_3$	0.67	206		
11	1-(3,7-Dimethyl-2-oxo-3,3a,4,5,8,8a-hexahydro-2H-cyclohepta[b]furan-6-yl)-3-oxobutyl acetate	$C_{17}H_{24}O_5$	0.39	308		
12	3,3a-Epoxydicyclopenta[a,d]cyclooctan-4β-ol, 9,10a-dimethyl-6-methylene-3β-isopropyl	$C_{20}H_{32}O_2$	0.35	308		

References

1. El-Sherbiny GM and Elbestawy MKM. A review – plant essential oils active against *Helicobacter pylori*. Journal of Essential Oil Research 2022; **34**203–215. doi: 10.1080/10412905.2022.2025464
2. Ribeiro-Santos R, Andrade M, Melo NR, and Sanches-Silva A. Use of Essential Oils in Active Food Packaging: Recent Advances and Future Trends. Trends Food Sci. Tech. 2017; **61**132–140. doi: 10.1016/j.tifs.2016.11.021
3. El-Sherbiny GM, Gazelly AM, Sharaf MH, Moghannemm SA, Shehata M, and other. Exploitation of the antibacterial, antibiofilm and antioxidant activities of Salvadora persica (Miswak) extract. A.S Journal of Bioresources and Bioproducts 2022 10.1016/j.jobab.2022.11.006
4. Ochoa-Velasco CE, Ávila-Sosa R, Navarro-Cruz AR, López-Malo A, and Palou E. Biotic and Abiotic Factors to Increase the Bioactive Compounds in Fruits and Vegetables. Chapter In Food Bioconversion 2017 317–348
5. Clemente I, Aznar M, Silva F, and Nerín C. Antimicrobial Properties and Mode of Action of Mustard and Cinnamon Essential Oils and Their Combination against Foodborne Bacteria. Innov. Food Sci. Emerg. Technol. 2016; **36**26–33. doi: 10.1016/j.ifset.2016.05.013
6. Sharma A, Flores-Vallejo RC, Cardoso-Taketa A, and Villareal ML. Antibacterial Activities of Medicinal Plants Used in Mexican Traditional Medicine. J. Ethnopharmacol 2017; **208**264–329. doi: 10.1016/j.jep.2016.04.045
7. Semwal RB, Semwal DK, Combrinck S, and Viljoen AM. Gingerols and Shogaols: Important Nutraceutical Principles from Ginger. Phytochemistry 2015; **117**554–568
8. Thongson C, Davidson PM, Mahakarnchanakul W, and Vibulsresth P. Antimicrobial Effect of Thai Spices against *Listeria Monocytogenes* and *Salmonella Typhimurium* DT104. J. Food Prot. 2005; **3**2054–2058
9. Kaushik P and Goyal P. Evaluation of Various Crude Extracts of *Zingiber Officinale* Rhizome for Potential Antibacterial Activity: A Study in Vitro. Adv. Appl. Microbiol. 2011; **1**7–12
10. Singh G, Maurya S, Catalan C, and De Lampasona MP. Studies on Essential Oils, , Part 42: Chemical, Antifungal, Antioxidant and Sprout Suppressant Studies on Ginger Essential Oil and Its Oleoresin. Flavour Fragr. J. 2005; **20**1–6. doi: 10.1002/ffj.1373
11. Figueroa-González G, Quintas-Granados LI, and other. Review of the anticancer properties of 6-shogaol: Mechanisms of action in cancer cells and future research opportunities. Food Science & Nutrition 2024; **12**4513–4533. doi: 10.1002/fsn3.4129
12. Hitomi S, Ono K, Terawaki K, Matsumoto C, and other. [6]-Gingerol and [6]-Shogaol, Active Ingredients of the Traditional Japanese Medicine Hangeshashinto, Relief Oral Ulcerative Mucositis-Induced Pain via Action on Na^+ Channels. Pharm. Res. 2017; **117**288–302. doi: 10.1016/j. phrs.2016.12.026
13. Soltani E, Jangjoo A, Aghaei AM, and Dalili A. Effects of Preoperative Administration of Ginger (Zingiber Officinale Roscoe) of Postoperative Nausea and Vomiting after Laparoscopic Cholecystectomy. J. Tradit. Complement. Med. 2017 1–4. doi: 10.1016/j.jtcme.2017.06.008
14. Chang JS, Wang KC, Yeh CF, Shieh DE, and Chiang LC. Fresh Ginger (Zingiber Officinale) Has Anti-Viral Activity against Human Respiratory Syncytial Virus in Human Respiratory Tract Cell Lines. J. Ethnopharmacol 2013; **145**145–151
15. Daily JW, Yang M, Kim DS, and Park S. Efficacy of Ginger for Treating Type 2 Diabetes: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. J. Ethn Foods 2015; **2**336–343
16. Elbestawy MKM, El-Sherbiny GM, Moghannem SA, and Farghal EE. Antibacterial, Antibiofilm, and Anti-Inflammatory Activities of *Ginger* Extract against *Helicobacter pylori*. Microbiology Research 2023; **14**1124–1138. doi: 10.3390/microbiolres14030075
17. Duarte MC, Tavares GSV, Valadares DG, Lage DP, Ribeiro TG, and other. Antileishmanial Activity and Mechanism of Action from a Purified Fraction of Zingiber Officinalis Roscoe against Leishmania Amazonensis. Exp. Parasitol. 2016; **166**21–28
18. Zhang F, Zhang JG, Qu J, Zhang Q, Prasad Q, and Wei ZJ. Assessment of Anti-Cancerous Potential of 6-Gingerol (Tongling White Ginger) and Its Synergy with Drugs on Human Cervical Adenocarcinoma Cells. Food Chem. Toxicol. 2017; **109**910–922
19. Beristain-Bauza SDC, Hernández-Carranza P, Cid-Pérez TS, Ávila-Sosa R, Ruiz-López II, and Ochoa-Velasco CE. Antimicrobial Activity of Ginger (*Zingiber Officinale*) and Its Application in Food Products. Food Reviews International 2019; **35**407–426. doi: 10.1080/87559129.2019.1573829
20. Jia Y, Li X, Meng X, Lei J, Xia Y, and Yu L. Anticancer perspective of 6-shogaol: anticancer properties, mechanism of action, synergism and delivery system. Chin Med. 2023; **18**138. doi: 10.1186/s13020-023-00839-0
21. Yeh IJ, Chen SC, Yen MC, Wu YH, Hung CH, and Kuo PL. 6-Shogaol suppresses 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP)-induced human 786-O renal cell carcinoma osteoclastogenic activity and metastatic potential. Nutrients 2019; **11**2306
22. Pei XD, He ZL, Yao HL, Xiao JS, Li L, and Gu JZ. 6-Shogaol from ginger shows anti-tumor effect in cervical carcinoma via PI3K/Akt/mTOR pathway. Eur J Nutr. 2021; **60**2781–93
23. Koshak AE et al. Antimicrobial and anti-virulence activities of 4-shogaol from grains of paradise against gram-negative bacteria: Integration of experimental and computational methods. J Ethnopharmacol 2024; **6**117611
24. Ok S and Jeong WS. Optimization of Extraction Conditions for the 6-Shogaol-rich Extract from Ginger (Zingiber officinale Roscoe). Prev Nutr Food Sci. 2012; **17**166–71. doi: 10.3746/pnf.2012.17.2.166
25. Bhattarai S, Tran VH, and Duke CC. The stability of gingerol and shogaol in aqueous solutions. J Pharm Sci. 2001; **90**1658–1664. doi: 10.1002/jps.1116
26. Foda AM, Kalaba MH, El-Sherbiny GM, Saad AM, and El-Fakharany EM. Antibacterial activity of essential oils for combating colistin-resistant bacteria. Expert Review of Anti-infective Therapy 2022. doi: 10.1080/14787210.2022.210199
27. Sharaf MH, El-Sherbiny GM, Moghannem SA, and other. New combination approaches to combat methicillin-resistant *Staphylococcus aureus* (MRSA). Sci Rep 2021; **11**1–16
28. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement. Wayne, PA: Clinical and Laboratory Standards Institute 2021; **6**
29. El-Sherbiny GM, Alluqmani AJ, Elsehemy IA, and other. Antibacterial, antioxidant, cytotoxicity, and phytochemical screening of *Moringa oleifera* leaves. Sci Rep 2024; **14**30485. doi: 10.1038/s41598-024-80700-y
30. Fareid MA. Impeding Biofilm-Forming Mediated Methicillin-Resistant *Staphylococcus aureus* and Virulence Genes Using a Biosynthesized Silver Nanoparticles–Antibiotic Combination. Biomolecules 2025; **15**266
31. Mastoor S, Nazim F, Rizwan-Ul-Hasan S, and other. Analysis of the Antimicrobial and Anti-Biofilm Activity of Natural Compounds and Their Analogues against *Staphylococcus aureus* Isolates. Molecules 2022; **27**6874. doi: 10.3390/molecules27206874
32. El-Sherbiny GM, Farghal EE, Lila MK, Shetaia YM, Mohamed SS, and Elswify MMF. Antibiotic susceptibility and virulence factors of bacterial species among cancer patients. Biotechnol Notes 2024 27–32
33. Shehata ME, El-Sherbiny GM, Sharaf MH, and other. Phytochemical analysis, antimicrobial, antioxidant, and cytotoxicity activities of *Schinus molle* (L.) extracts. Biomass Conv. Bioref. 2024. doi: 10.1007/s13399-024-05301-1
34. Elbestawy MKM, El-Sherbiny GM, and Moghannem SA. Antibacterial, Antibiofilm and Anti-Inflammatory Activities of Eugenol Clove Essential Oil against Resistant *Helicobacter pylori*. Molecules 2023; **28**2448
35. Saravanan S and Parimelazhagan T. *In vitro* antioxidants, antimicrobial and anti-diabetic properties of polyphenols of *Passiflora ligularis* Juss. fruit pulp. Food Science and Human Wellness 2014; **3**5664
36. Apostolidis E and Lee C. *In vitro* potential of *Ascophyllum nodosum* phenolic antioxidant-mediated α-glucosidase and α-amylase inhibition. J. Food Sci. 2010; **75**H97–H102
37. El-Sherbiny GM et al. Nanoemulsion of cinnamon oil to combat colistin-resistant Klebsiella pneumoniae and cancer cells. Microb Pathog. 2024; **192**106705. doi: 10.1016/j.micpath.2024.106705
38. Alghazzaly AM, El-Sherbiny GM, Moghannemm SA, Sharaf MH, and Abd ElAziz AA. Antibacterial, Antibiofilm, Antioxidants and Phytochemical Profiling of *Syzygium aromaticum* Extract. Egyptian Journal of Aquatic Biology & Fisheries 2022; **26**207–218
39. Ersedo TL et al. Food flavor enhancement, preservation, and biofunctionality of ginger (*Zingiber officinale*): a review. International Journal of Food Properties 2023; **26**928–951

40. Edo GI, Ugbune U, Akpoghelie PO, and other. Evaluation of physicochemical, phytochemical, anti-bacterial and antioxidant potential of kola nut (*cola acuminata*): an approach in food, health and nutritional benefits. Vegetos 2024; **37**2320–2329. doi: 10.1007/s42535-023-00715-0
41. Shareef HK, Muhammed HJ, Hussein HM, and Hameed IH. Antibacterial Effect of Ginger (*Zingiber officinale*) Roscoe and Bioactive Chemical Analysis using Gas Chromatography Mass Spectrum. Oriental Journal of Chemistry 2016; **32**817–837
42. Liu Q, Meng X, Li Y, Zhao C, Tang G, and Li H. Antibacterial and Antifungal Activities of Spices. Int. J. Mol. Sci. 2017; **18**61283. doi: 10.3390/ijms18061283
43. Mukkavilli R, Yang C, Singh Tanwar R, Ghareeb A, Luthra L, and Aneja R. Absorption, Metabolic Stability, and Pharmacokinetics of Ginger Phytochemicals. Molecules 2017; **22**4553. doi: 10.3390/molecules22040553
44. Sellami M, Ghariani B, Louati H, Miled N, and Gargouri Y. Biological Activities of Extracts of Different Spices and Plants. Int. J. Curr. Eng. Technol. 2013; **3**31051–1060
45. Hasan HA, Rashhed Raauf AM, Abd Razik BM, and Rasool Hassan BA. Chemical Composition and Antimicrobial Activity of the Crude Extracts Isolated from Zingiber Officinale by Different Solvents. Pharmaceut. Ana. Acta. 2013; **3**1–5
46. Singh G, Fapoor IPS, Shigh P, Heluani CS, Lampasona MP, and Catalan CAN. Chemistry, Antioxidant and Antimicrobial Investigations on Essential Oil and Oleoresins of Zingiber Officinale. Food Chem. Toxicol. 2008; **46**3295–3302. doi: 10.1016/j.fct.2008.07.017.2435.1000184
47. Koshak AE et al. Antimicrobial and anti-virulence activities of 4-shogaol from grains of paradise against gram-negative bacteria: Integration of experimental and computational methods. J Ethnopharmacol 2024; **6**. doi: 10.1016/j.jep.2023.117611
48. Mathai K, Anand S, Aravind A, Dinatius P, Krishnan AV, and Mathai M. Antimicrobial Effect of Ginger, Garlic, Honey, and Lemon Extracts on Streptococcus mutans. J Contemp Dent Pract. 2017; **1**1004–1008. doi: 10.5005/jp-journals-10024-2165
49. Yusuf AA, Lawal B, Abubakar AN, and other. *In-vitro* antioxidants, antimicrobial and toxicological evaluation of Nigerian *Zingiber officinale*. Clin Phytosci 2018; **4**12. doi: 10.1186/s40816-018-0070-2
50. Chakraborty B, Nath A, Saikia H, and Sengupta M. Bactericidal activity of selected medicinal plants against multidrug resistant bacterial strains from clinical isolates. Asian Pac J Trop Med. 2014. doi: 10.1016/S1995-7645(14)60271-6
51. Aghazadeh M et al. Survey of the Antibiofilm and Antimicrobial Effects of Zingiber officinale (in Vitro Study). Jundishapur J Microbiol 2016; **9**e30167. doi: 10.5812/jjm.30167
52. Chakotiya AS, Tanwar A, Narula A, and Sharma RK. Zingiber officinale: Its antibacterial activity on *Pseudomonas aeruginosa* and mode of action evaluated by flow cytometry. Microb. Pathogenesis 2017; **107**254–260
53. Kim H and Park H. Ginger extract inhibits biofilm formation by Pseudomonas aeruginosa PA14. PLoS ONE 2013; **8**e76106
54. Hasan S, Danishuddin M, and Khan AU. Inhibitory effect of Zingiber officinale towards Streptococcus mutans virulence and caries development: in vitro and in vivo studies. BMC Microbiol. 2015; **15**1
55. Rampogu S et al. Ginger (*Zingiber officinale*) phytochemicals-gingerenone-A and shogaol inhibit SaHPPK: molecular docking, molecular dynamics simulations and in vitro approaches. Ann. Clin. Microb. Anti. 2018; **17**16
56. Lee JH, Kim YG, Choi P, Ham J, Park JG, and Lee J. Antibiofilm and Antivirulence Activities of 6-Gingerol and 6-Shogaol Against *Candida albicans* Due to Hyphal Inhibition. Front Cell Infect Microbiol. 2018. doi: 10.3389/fcimb.2018.00299
57. Mao QQ et al. Bioactive Compounds and Bioactivities of Ginger (*Zingiber officinale* Roscoe). Foods 2019; **8**185. doi: 10.3390/foods8060185
58. Abolaji AO, Ojo M, Afolabi TT, Arowoogun MD, Nwawolor D, and Farombi EO. Protective properties of 6-gingerol-rich fraction from Zingiber officinale (ginger) on chlorpyrifos-induced oxidative damage and inflammation in the brain, ovary and uterus of rats. Chem. Biol. Interact. 2017; **270**15–23
59. Li Y, Hong Y, Han Y, Wang Y, and Xia L. Chemical characterization and antioxidant activities comparison in fresh, dried, stir-frying and carbonized ginger. J. Chromatogr. B 2016; **1011**223–232
60. Sakulnarmrat K, Srzednicki G, and Konczak I. Antioxidant, enzyme inhibitory and antiproliferative activity of polyphenolic-rich fraction of commercial dry ginger powder. Int. J. Food Sci. Tech. 2015; **50**2229–2235
61. Gunathilake KDPP and Rupasinghe HPV. Inhibition of human low-density lipoprotein oxidation in vitro by ginger extracts. J. Med. Food 2014; **17**424–431
62. Upadhyaya S, Yadav D, Chandra R, and Arora N. Evaluation of Antibacterial and Phytochemical Properties of Different Spice Extracts. Afr. J. Microbiol. Res. 2018; **12**27–37. doi: 10.5897/AJMR2017.8731
63. Si W, Chen YP, Zhang J, Chen ZY, and Chung HY. Antioxidant Activities of Ginger Extract and Its Constituents Toward Lipids. Food Chem. 2018; **239**1117–1125. doi: 10.1016/j.foodchem.2017.07.055
64. Zhang M et al. Edible ginger-derived nanoparticles: A novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer. Biomaterials 2016; **101**321–340
65. Zhang G, Nitteranon V, Chan LY, and Parkin KL. Glutathione conjugation attenuates biological activities of 6-dehydroshogaol from ginger. Food Chem. 2013; **140**1–8
66. Luettig J, Rosenthal R, Lee IM, Krug SM, and Schulzke JD. The ginger component 6-shogaol prevents TNF-alpha-induced barrier loss via inhibition of PI3K/Akt and NF-kappa B signaling. Mol. Nutr. Food Res. 2016; **60**2576–2586
67. Hsiang C, Lo H, Huang H, Li C, Wu S, and Ho T. Ginger extract and zingerone ameliorated trinitrobenzene sulphonic acid-induced colitis in mice via modulation of nuclear factor-kappa B activity and interleukin-1 beta signalling pathway. Food Chem. 2013; **136**170–177
68. Ueno N et al. TU-100 (Daikenchuto) and ginger ameliorate anti-CD3 antibody induced T cell-mediated murine enteritis: microbe-independent effects involving Akt and Nf-kappa b suppression. PLoS ONE 2014; **9**e97456
69. Zhu Y, Zhao Y, Wang P, Ahmedna M, and Sang S. Bioactive ginger constituents alleviate protein glycation by trapping methylglyoxal. Chem. Res. Toxicol 2015; **28**1842–1849
70. Sampath C, Rashid MR, Sang S, and Ahmedna M. Specific bioactive compounds in ginger and apple alleviate hyperglycemia in mice with high fat diet-induced obesity via Nrf2 mediated pathway. Food Chem. 2017; **226**79–88
71. Bin Samad M et al. [6]-Gingerol, from Zingiber officinale, potentiates GLP-1 mediated glucose-stimulated insulin secretion pathway in pancreatic beta-cells and increases RAB8/RAB10-regulated membrane presentation of GLUT4 transporters in skeletal muscle to improve hyperglycemia in Lepr(db/db) type 2 diabetic mice. BMC Complem. Altern. M. 2017; **17**395
72. Arablou T, Aryaeian N, Valizadeh M, Sharifi F, Hosseini A, and Djalali M. The effect of ginger consumption on glycemic status, lipid profile and some inflammatory markers in patients with type 2 diabetes mellitus. Int. J. Food Sci. Nutr. 2014; **65**515–520
73. Li Y, Tran VH, Kota BP, Nammi S, Duke CC, and Roufogalis BD. Preventative effect of Zingiber officinale on insulin resistance in a high-fat high-carbohydrate diet-fed rat model and its mechanism of action. Basic Clin. Pharmacol. 2014; **115**209–215
74. Dongare S et al. Zingiber officinale attenuates retinal microvascular changes in diabetic rats via anti-inflammatory and antiangiogenic mechanisms. Mol. Vis. 2016; **22**599–609
75. Mahluji S, Attari VE, Mobasseri M, Payahoo L, Ostadrahimi A, and Golzari SEJ. Effects of ginger (Zingiber officinale) on plasma glucose level, HbA1c and insulin sensitivity in type 2 diabetic patients. Int. J. Food Sci. Nutr. 2013; **64**682–686
76. Bray F et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024; **74**229–263. doi: 10.3322/caac.21834
77. Figueroa-González G, Quintas-Granados LI, Reyes-Hernández OD, Caballero-Florán IH, and other. Review of the anticancer properties of 6-shogaol: Mechanisms of action in cancer cells and future research opportunities. Food Sci Nutr. 2024; **12**4513–4533. doi: 10.1002/fsn3.4129
78. Sakulnarmrat K, Srzednicki G, and Konczak I. Antioxidant, enzyme inhibitory and antiproliferative activity of polyphenolic-rich fraction of commercial dry ginger powder. Int. J. Food Sci. Tech. 2015; **50**2229–2235

79. Tahir AA, Sani NFA, Murad NA, Makpol S, Ngah WZW, and Yusof YAM. Combined ginger extract & Gelam honey modulate Ras/ERK and PI3K/AKT pathway genes in colon cancer HT29 cells. Nutr. J. 2015; **14**31
80. Deol PK and Kaur IP. Improving the therapeutic efficiency of ginger extract for treatment of colon cancer using a suitably designed multiparticulate system. J. Drug Target 2013; **21**855–865
81. Zhang F, Zhang J, Qu J, Zhang Q, Prasad C, and Wei Z. Assessment of anti-cancerous potential of 6-gingerol (Tongling white ginger) and its synergy with drugs on human cervical adenocarcinoma cells. Food Chem. Toxicol. 2017; **109**910–922
82. Tao Y, Li W, Liang W, and Van Breemen RB. Identification and quantification of gingerols and related compounds in ginger dietary supplements using high-performance liquid chromatography-tandem mass spectrometry. J Agric Food Chem. 2009; **57**10014–21. doi: 10.1021/jf9020224
83. Semwal RB, Semwal DK, Combrinck S, and Viljoen AM. Gingerols and Shogaols: Important Nutraceutical Principles from Ginger. Phytochemistry 2015; **117**554–568. doi: 10.1016/j.phytochem.2015.07.012
84. Choi JG, Kim SY, Jeong M, and Oh MS. Pharmacotherapeutic Potential of Ginger and Its Compounds in Age-Related Neurological Disorders. Pharmacol. Ther. 2018; **182**56–69. doi: 10.1016/j.pharmthera.2017.08.010
85. Chen CC, Kuo MC, Wu CM, and Ho CT. Pungent Compounds of Ginger (Zingiber Officinale Roscoe) Extracted by Liquid Carbon Dioxide. J. Agric. Food Chem. 1986; **34**477–480. doi: 10.1021/jf00069a027
86. Schadich E, Hlavac J, Volna T, Varanasi L, Hajduch M, and Dzubak P. Effects of ginger phenylpropanoids and quercetin on Nrf2-ARE pathway in human BJ fibroblasts and HaCaT keratinocytes. Biomed Res. Int. 2016; **2016**2173275