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ORIGINAL ARTICLE

Antibiofilm and Anti-Adhesive Effects of Ginger against Some Food-Related Pathogens

Ghada M. Khiralla*

Division of Medical Foods, National Organization for Drug Control and Research (NODCAR), 6-7, AboHazem St, Pyramids, Giza, Egypt.

*Corresponding Author:

Ghada M. Khiralla

Email: gkhiralla@yahoo.com

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Abstract

Ginger have been used in folk medicine as a hot or cold water extract. The present study aimed at evaluating the antimicrobial, anti-adhesive and antibiofilm potential of fresh and cooked ginger water extracts (FGE and CGE, respectively). The main bioactive components were 6-, 8-, and 10- gingerols in FGE, and 6-, 8-, and 10- shogaols in CGE. Both extracts showed antimicrobial effect against six strains of *Salmonella* Typhimurium (3 strains) and *Escherichia coli* O157:H7 (3 strains). MIC_{50} of FGE and CGE against both studied groups was 25 mg DTSS /mL, whereas MIC_{90} was not recorded. Comparing to CGE, FGE displayed high potential anti-adhesive and antibiofilm *in vitro*. Use of 20 mg DTSS /mL of FGE led to reduce the adhesive of bacterial cells to HEp-2 cells by about 50%. In conclusion, soaking 4-5g fresh ginger rhizome in 200 mL cold water (20–25 DTSS) for about 12h could be suggested to prepare ginger water extract with desired anti-adhesive and antibiofilm potential against the food related pathogens such as Gram negative *S.* Typhimurium and *E. coli* O157:H7.

Key words: Ginger, Biofilm, Adhesion, *Salmonella* Typhimurium, *Escherichia coli*.

1. Introduction

Salmonella Typhimurium and *Escherichia coli* are Gram-negative bacteria that cause various gastrointestinal diseases. *E. coli* O157:H7 is the most important enterohaemorrhagic *E. coli* (EHEC) serotype in relation to public health (Lim *et al.*, 2010). *E. coli* O157:H7 is well known as a major Shiga toxin-producing food-borne pathogen that has a low infectious dose and causes hemorrhagic colitis, hemolytic uremic syndrome, and even death (Melton-Celsa *et al.*, 2012; Xue *et al.*, 2014). *E. coli* O157:H7 interaction with intestinal epithelial cells is the first step in its colonization in the host cells. *E. coli* O157:H7 possesses several effectors that allow it to familiarly adhere to intestinal epithelial cells (Frankel and Phillips, 2008; Melton-Celsa *et al.*, 2012).

In general, bacterial adhesion to host cells, which is an important virulence factor, plays an important role in gastrointestinal infectious disease (Kalliomaki and Walker, 2005). After initial adhesion to host tissues, bacteria begin to grow as a monolayer on the surface to form microcolonies that can consequently develop and form biofilm. A biofilm may be described as a microbial-derived sessile community characterized by cells that adhere to an interface, embedded in a matrix. Depending on the species involved, microcolonies may be composed of 10–25% cells and 75–90% extracellular polymeric substances (EPS) matrix (Garrett *et al.*, 2008). Presence of pathogenic bacteria in biofilm matrix increases their virulence and resistance to disinfectants and antibiotics (Wojnicz *et al.*, 2012). Adhesion of bacteria and consequently biofilm formation is managed by several factors such as electrostatic interactions, van der Waals, acid–base, hydrogen bonding, biospecific interactions (Fletcher, 1996), bacterial surface roughness, solid surface chemical structure (Abu-Lail and Camesano, 2003) and hydrophobicity (Elhariry, 2008). All these surface structures and properties are well known to be affected by treatment with plant extracts rich in phenolic compounds and isoflavones (Huttunen *et al.*, 2011; Elhariry *et al.*, 2014). Plant extracts could be considered as promise alternative nonantibiotic strategy aimed to reduce the production of virulence factors without affecting bacterial growth, which may be less prone to develop drug resistance (Cegelski *et al.*, 2009).

Ginger (*Zingiber officinale*) is one of the most frequently used ingredients in folk medicine and cooking. Ginger rhizome contains two classes of constituents; the essential oils, which give the aroma, and 6-, 8-, and 10- gingerols, which considered as main pungent principles (Young *et al.*, 2002). During thermal processing or storage, the gingerols may be modified to a series of homologous compounds of shogaols such as 6-, 8-, and 10-shogaol (Sang *et al.,* 2009; Shao *et al.,* 2010). Various *in vitro* and *in vivo* studies have been carried out on the pharmacological potential of ginger extract including preventing blood clots, reducing cholesterol and triglyceride levels, and increasing high-density lipoprotein (Bhandari *et al.,* 2005; Nammi *et al.,* 2009; Rehman *et al.*, 2011). Essential oils (ethanolic extract) of ginger have been studied for their antimicrobial activity against different bacteria including *E. coli, S.* Typhi (Meliani *et al.*, 2014; Maekawa *et al.,* 2015). Moreover, essential oils of ginger have been used for preventing biofilm formation by *Pseudomonas aeruginosa* PA14 (Kim and Park, 2013) *Staphylococcus aureus* (Lee *et al.*, 2014) and *Candida albicans* (Agarwal *et al.*, 2008), *E. coli* ATCC 25922, but did not show antibiofilm effect against *S.* Typhimurium (Nikolić *et al.*, 2014). For the best of the author's knowledge, ginger water extract has not been studied for its anti-adhesive and antibiofilm potential against pathogenic bacteria, especially *S.* Typhimurium and *E*. coli O157:H7. To avoid toxicity and possible negative effects caused by essential oils and ethanol extracts (Wang *et al.,* 2012), distilled water was used as the extraction agent in the present study. Therefore, the present study aimed to (i) determine the bioactive compounds in the ginger water extract including gingerols and shogaols, and (ii) evaluate antimicrobial, anti-adhesive and antibiofilm effect of ginger water extract against three strains of *S.* Typhimurium and three strains of *E. coli* O157:H7; where blocking bacterial adhesion to host surfaces provides potential approach to control the microbial infections.

2. Materials and Methods

2.1 Bacterial Strains

Six pathogenic bacteria were used in the present study, including *Salmonella enterica* serovar Typhimurium ATCC 19585, ATCC 14028 and ATCC 23564 and *Escherichia coli* O157:H7 strain ATCC 43895, ATCC 35150 and ATCC 43894. These bacteria were obtained from stock cultures maintained in the microbial culture collection, Department of Food Science, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. All strains were cultivated routinely on tryptic soy broth (TSB) for overnight at 37 °C and stored at 4 °C on tryptic soy agar (TSA). Stock cultures were maintained in 15% v/v glycerol/TSB at -70 °C.

2.2 Preparing Ginger Water Extract

Ginger rhizome were purchased from the local market, Giza City, Egypt, and used for preparing ginger water extract. In the present study, two extraction procedures were designed to simulate the common methods used for consumption of ginger at home level. Ginger rhizomes were thoroughly washed under tap water and cut into small pieces. 200 g ginger rhizome pieces were minced in a home blender with 200 mL water for 5 min. For preparing fresh ginger extract (FGE), the final volume was adjusted to 500 mL using distilled water and left for 12 h at 27 ºC. For preparing cooked ginger extract (CGE), the ginger rhizome blend was heated to boiling point (~ 100 ºC) for 15 min and then allowed to cooling to room temperature. The final volume was adjusted to 500 mL using distilled water. FGE and CGE were filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). To obtain the dried total soluble solids (DTSS) of these extracts, the filtrates were dried in at 37 °C for 48 h (Elhariry *et al.*, 2014) and expressed as mg DTSS/g fresh ginger rhizome (w/w). The DTSS was used for determination of bioactive compounds and for studying anti-adhesive and antibiofilm effects against some food-related bacterial pathogens.

2.3 Determination of Bioactive Compound

2.3.1 Total Phenols

The total phenolic content of ginger extracts (FGE and CGE) was determined according to the method described by Amarowicz *et al.* (2004). Briefly, stock extracts (FGE and CGE) contained 100 mg DTSS/mL were filtrated through 0.22 µm filters. 18 µL of each extract was transferred into a 96-well microtiter plate (Biotek, Winooski, VT, USA) and mixed with 36 µL Folin–Ciocalteu reagent (10%) and 145 µL sodium carbonate (700 mmoL). The absorbance of the mixture was measured at 750 nm after 60 min incubation in the dark at room temperature. The total phenolic content was calculated as a gallic acid equivalent (GAE) from a calibration curve of GA standard solutions (25-800 µg/mL) and expressed as mg GAE /g DTSS. All measurements were done in triplicate (*n=3*).

2.3.2 HPLC Analysis of Gingerols and Shogaols

The filtered ginger extracts (FGE and CGE) were subjected to HPLC system (Schimadzu, LC10AT, Nakagyo-ku, Kyoto, Japan) equipped with a Luna 2.5

μm C18(2) HST (100 x2.0 mm). Isocratic column elution was monitored by 1200 UV/visible detector at wavelength of 282 nm. The mobile phase was consisted of water (A) and Acetonitrile (B) (HPLC-grade, Sigma, USA). Gradient elution A/B was adjusted to be 60/40 at 1 min, 20/80 at 9 min, 5/95 at 10 min and then hold at this gradient for 3 min. The flow rate was 0.5 mL/min and the column temperature was 50°C. Reference chemicals, 6-, 8-, 10-gingerol, 6-, 8-, and 10-shogaol (ChromaDex, Santa Ana, CA, USA) were used as standards for calibration. All standards were dissolved in methanol:water (80:20) and filtered through a 0.22 μm filters before injection in HPLC system.

2.3.3 Antimicrobial Activity

Antimicrobial activity of FGE and CGE was determined as minimum inhibitory concentration (MIC_{50} or MIC_{90}) using a micro-dilution technique in 96-well microtiter plates (Eloff, 1998). Since 1g of fresh ginger rhizome contained about 5 mg DTSS, and the consumer can prepare ginger extract using about 3-5 g fresh ginger per 200 mL (cup), the studied concentrations were in the range of 15-25 mg DTSS. To achieve these concentrations, stock extracts (FGE and CGE) contained 100 mg DTSS/mL were sterilized by filtration through 0.22 μm filters. Different amounts; 25, 37.5, 50, 62.5 and 75 μL of the concentrated stock of FGE and CGE were added to each well of sterile 96-well polystyrene plates. 10 μL activated culture of each tested strain (about 10^6 CFU/mL) were added. The total volume in each well was adjusted to 250 μL using TSB. The final tested concentrations of DTSS were 10, 15, 20, 25 and 30 mg DTSS/mL. After 18 h of incubation at 37 ºC, bacterial growth inhibition was determined by monitoring the optical density (OD) at 595 nm. The MIC_{50} and MIC_{90} were defined as the lowest concentration of DTSS of ginger-extract inhibiting >50% and >90% of bacterial growth, respectively. All tests were carried out in triplicate (*n=3*) and the results were averaged.

2.3.4 Anti-adhesive and Antibiofilm Effect

Inhibitory effects of the studied ginger extracts (FGE and CGE) on bacterial adhesion and biofilm formation were determined *in vitro* using the commonly used 96 wells polystyrene microtiter-plates method (Elhariry *et al.*, 2014). The steps of the first part of this experiment were done as described above in determination of antimicrobial activity. After incubation at 37 ºC for 4 (to determine anti-adhesive effect) and 24 h (to determine antibiofilm effect), content of the microtiter plates were poured off and the wells were washed three times with 300 μL of phosphate-buffered saline (PBS, pH 7.2). The remaining adhered bacteria were fixed with 250 μL of methanol per well. After 15 min microtiter plates were emptied and air dried. The microtiter plates were stained with 250 μL per well of 1% crystal violet used for Gram staining for 5 min. The excess of stain were rinsed off by placing the microtiter plates under running tap water. After drying the microtiter plates, the dye bound to the adherent cells were extracted with 250 μL of 33% (v/v) glacial acetic acid per well. The absorbance of each was measured at 570 nm using a spectrophotometer. Based on the absorbance (A_{570nm}) produced by bacterial films, strains were classified into four categories according to the classification of Christensen *et al.* (1985) which modified by Stepanovic *et al.* (2000). Briefly, the cut-off absorbance (*A*c) was the mean absorbance of the negative control (wells with 250 μL TSB only). Strains were classified as follows: $A = Ac$ = no biofilm producer (-); $Ac < A \leq (2 \times Ac)$ = weak biofilm producer (+); $(2 \times Ac) < A \leq (4 \times Ac)$ = moderate biofilm producer (++); $(4 \times Ac) < A$ = strong biofilm producer (+++). All tests were carried out in triplicate (*n=3*) and the results were averaged.

The anti-adhesive effect was calculated from the absorbance values after 4h. Here, the anti-adhesive dose was considered as the minimum concentration of ginger extract required for complete prevention of bacterial cell adhesion to microtiter-plates substratum, when $A = Ac$ after 4h. The antibiofilm dose was defined here as the lowest DTSS concentration of ginger extract causing preventing biofilm formation after 24h by each tested bacterium (to be in the category of no biofilm producer).

2.3.5 Bacterial Adherence to HEp-2 Cells and Anti-adhesive Effect

The adherence assay was carried out according to the method described by Boddicher *et al.* (2002) and modified by (Elhariry *et al.,* 2014). Briefly, washed HEp-2 cells were incubated with bacteria (1×10^8 CFU/mL) in a bacteria to cell ratio of 1000:1 in the absence (control) or presence of ginger extracts (FGE and CGE). Following incubation with gentle agitation non-adherent organisms were removed by repeated washing. After a final wash the cells were resuspended in 200 μL of PBS (pH 7.2) and fixed onto glass microscope slides. After staining with Giemsa the cells were examined by microscopy and adherence was recorded as the mean number of bacteria per cell after examination of at least 100 different cells from five separate fields (Boddicker *et al.,* 2002). All tests were carried out in triplicate (*n=3*) and the results

Table 1: Bioactive compounds of fresh (FGE) and cooked (CGE) ginger extracts

Compounds [*]	FGE[#]	CGE[#]
DTSS[$] (mg/g)	5.06 [a] ± 0.21	5.21 [a] ± 0.17
Total phenols[&] (µg/mg)	32.73 [a] ± 1.13	27.93 [b] ± 1.18
Total gingerols[@] (µg/mg)	22.11[a] ± 1.24	11.16 [b] ± 1.23
6-gingerol	11.22 [a] ± 0.61	6.23 [b] ± 0.51
8-gingerol	5.72 [a] ± 0.34	2.71 [b] ± 0.94
10-gingerol	5.17 [a] ± 1.03	2.22 [b] ± 0.74
Total shogaols[@] (µg/mg)	10.23 [b] ± 1.66	15.29 [a] ± 1.02
6-shogaol	6.77 [b] ± 0.28	9.88[a] ± 0.93
8-shogaol	1.05 [b] ± 0.42	1.79 [b] ± 0.68
10-shogaol	2.41 [b] ± 0.53	3.62 [a] ± 0.14

[*] *were determined by HPLC, values are means ±SD of three independent experiments (n=3).*
[#] *ginger extracts (20% w/v) prepared by soaking in cold-water and named fresh ginger extract (FGE) or by boiling in water and named cooked ginger extract (CGE).*
[$] *DTSS is the dried total soluble solids expressed per 1 g fresh ginger rhizome (w/w).*
[&] *expressed as µg GAE /mg DTSS.*
[@] *xpressed as µg bioactive compound / mg DTSS.*
Values in the same row with the same letter are insignificantly differed, p=0.05.

Table 2: Antibiofilm activity of ginger water extracts against some *S.* Typhimurium strains and *E. coli* O157:H7.

Strains	Biofilm formation[a]				
	FGE[c] (mg DTSS[b] /mL)				
	0	10	15	20	25
S. Typhimurium					
ATCC 19585	+++	++	+	-	-
ATCC 14028	++	+	-	-	-
ATCC 23564	+++	++	+	-	-
Escherichia coli O157:H7					
ATCC 43895	+++	++	++	-	-
ATCC 35150	+++	++	+	-	-
ATCC 43894	+++	++	+	-	-
Strains	CGE[c] (mg DTSS[b] /mL)				
	0	10	15	20	25
S. Typhimurium					
ATCC 19585	+++	++	++	+	-
ATCC 14028	++	++	++	+	-
ATCC 23564	+++	++	++	+	-
Escherichia coli O157:H7					
ATCC 43895	+++	++	++	+	-
ATCC 35150	+++	++	++	+	-
ATCC 43894	+++	++	++	+	-

[a] *Strains were classified as no biofilm producer (-), weak biofilm producer (+), moderate biofilm producer (++) or strong biofilm producer (+++).*
[b] *DTSS is the dried total soluble solids expressed per 1 g fresh ginger rhizome (w/w).*
[c] *FGE and CGE are fresh and cooked ginger water-extract respectively; which were prepared by dissolving per 1 mL: 0, 10, 15, 20, or 25 mg DTSS.*

were averaged. Here, the reduction percentage of adhesive was calculated as [(number of adhered bacteria per HEp-2 cell in the presence of ginger extract - number of adhered bacteria per HEp-2 cell of control (in the absence of ginger extract)] / number of adhered bacteria per HEp-2 cell of control.

2.4 Statistical Analysis

The data obtained from three replicates were analyzed by a one-way ANOVA (SAS 8.2, Cary, NC, USA) to illustrate the effect of different ginger-extract concentration on each studied strain. In all cases, the level of statistical significance was of $p < 0.05$.

3. Results and Discussion

Different extraction methods have been developed in previous studies to maximize the bioactive components in ginger extracts. However, in the present study, ginger water extracts were prepared according to the common methods that applied by the popular consumers using hot and cold water. Two ginger water extracts were prepared: fresh ginger extract (FGE) by soaking in cold water for 12h and cooked ginger extract (CGE) using hot water (~ 100 ºC) for 15 min. Dry total soluble solids (DTSS, mg/g fresh ginger) of FGE and CGE were determined and used for preparation of studied concentrations. No significant ($p>0.05$) difference was recorded in the DTSS of both FGE and CGE (5.06 ±0.21 and 5.21 ±0.17 mg/g, respectively).

3.1 Bioactive Compounds of Ginger Water Extracts

The FGE had a high phenolic content (32.73 ±0.13 µg GAE /mg DTSS) compared with that of CGE (Table 1). This result indicated that the phenolic content of ginger may be partially lost by exposure to high temperature during extraction. Kishk and Sheshetawy (2010) have mentioned that total phenols of the ginger extract prepared at 22 °C were higher than those obtained at 100 °C. The well-known bioactive compounds including gingerols and shogaols were determined in both extracts using HPLC (Table 1). FGE displayed significant ($p<0.05$) increase in its content of 6-, 8- and 10-gingerol compared with that of CGE. On the other hand, CGE showed significant ($p<0.05$) high level of 6-, and 10-shogaol compared with that of FGE, whereas insignificant ($p>0.05$) difference was noticed in the 8-shogaol content of both extracts (Table 1). In general, FGE was characterized by its high content of total gingerols (22.11 ±1.24 µg/ mg DTSS) compared with CGE (11.16 ±1.23 µg/ mg DTSS), which had high content of total shogaols (15.29 ±1.02 µg/ mg DTSS). This result pointed to the partial conversion of gingerols to shogaols due to exposure to heat during preparation of CGE. In accordance with the obtained results, the gingerols have been identified in the fresh ginger rhizome as the major active components, and 6-gingerol is the most abundant constituent in the gingerols (Baranowski, 1985). Moreover, Bhattarai *et al.* (2001) demonstrated that in the aqueous solution, rapid degradation of gingerol mainly occurs at high temperature and at an acidic pH, resulting in dehydration to form shogaol.

3.2 Antimicrobial Activity of Ginger Water Extracts

Different DTSS concentrations of FGE and CGE (0, 10, 15, 20, 25 and 30 mg/mL) were tested for its antimicrobial activity against six food-related pathogens (Fig 1). The inhibitory effect of FGE against all tested strains was increased by increasing DTSS concentration. Concerning CGE, no significant effect of 10 mg/mL was noticed against *S.* Typhimurium strains, however the growth of E. *coli* O157: H7 strains was significantly reduced. More than 50% reduction in the growth of all tested strains were recorded when 25 mg DTSS /mL of both FGE and CGE were added to the medium. Moreover, no significant reduction in the growth of tested strains was noticed when 30 mg DTSS /mL was tested. Therefore, 25 mg DTSS /mL of both FGE and CGE were considered here as MIC_{50} against *S.* Typhimurium and *E. coli* O157:H7. This means that, to obtain the antimicrobial effect of fresh ginger water-extract (either fresh or cooked) man should use at least 5 g fresh ginger per 200 mL for preparing an effective hot or cold ginger drink. However, use of ginger by this procedure did not guaranty complete inhibition of the studied bacterial pathogens, where MIC_{90} was not recorded. Results of the present work indicated that, the MIC_{50} of FGE and CGE was similar, although, there are significant differences in the level of bioactive compounds in both extracts. This indicated that the synergistic effect of bioactive components in both tested extracts was similar. In accordance with the obtained results, complete inhibition of *S.* Typhimurium or *E. coli* has not been recorded in the presence of ginger water extract (Indu *et al.,* 2006).

3.3 Anti-adhesive and Antibiofilm Effects of Ginger Water Extracts

Adhesion and biofilm formation of *S.* Typhimurium strains and *E. coli* O157: H7 were *in vitro* investigated using 96-wells microtiter plate technique in the absence and presence of ginger water-

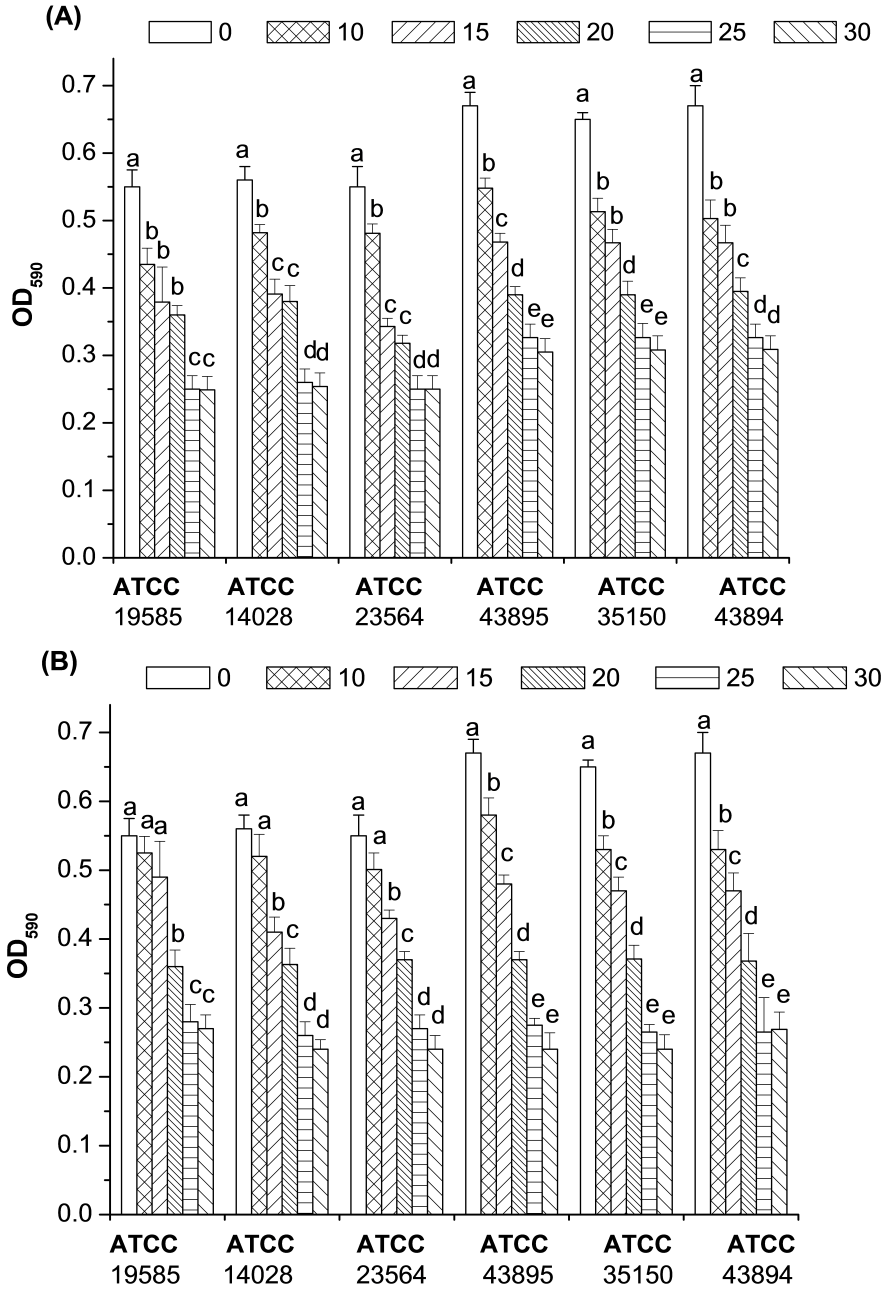

Fig 1**:** Antimicrobial effect of fresh (A) and cooked (B) ginger water extracts against *S.* Typhimurium strains ATCC 19585, ATCC 14028 and ATCC 23564 and *E. coli* O157:H7 strains ATCC 43895, ATCC 35150 and ATCC 43894. Tested concentrations were prepared by dissolving per 1 mL: 0, 10, 15, 20, 25 or 30 mg of the dried total soluble solids (DTSS) of FGE and CGE. Values are means ±SD, *n = 3*. Columns with the same letter within each group are insignificantly different (*P > 0.05*).

extracts (FGE and CGE). In the absence of ginger extracts (positive control), all studied strains showed high ability to adhere to polystyrene substratum (Fig 2). Both tested extracts impaired but did not prevent adhesion of all tested strains when the extracts were used at concentration of 10 and 15 mg DTSS/ mL. In general adhesion of all studied strains was reduced by increasing the concentration of FGE and CGE.

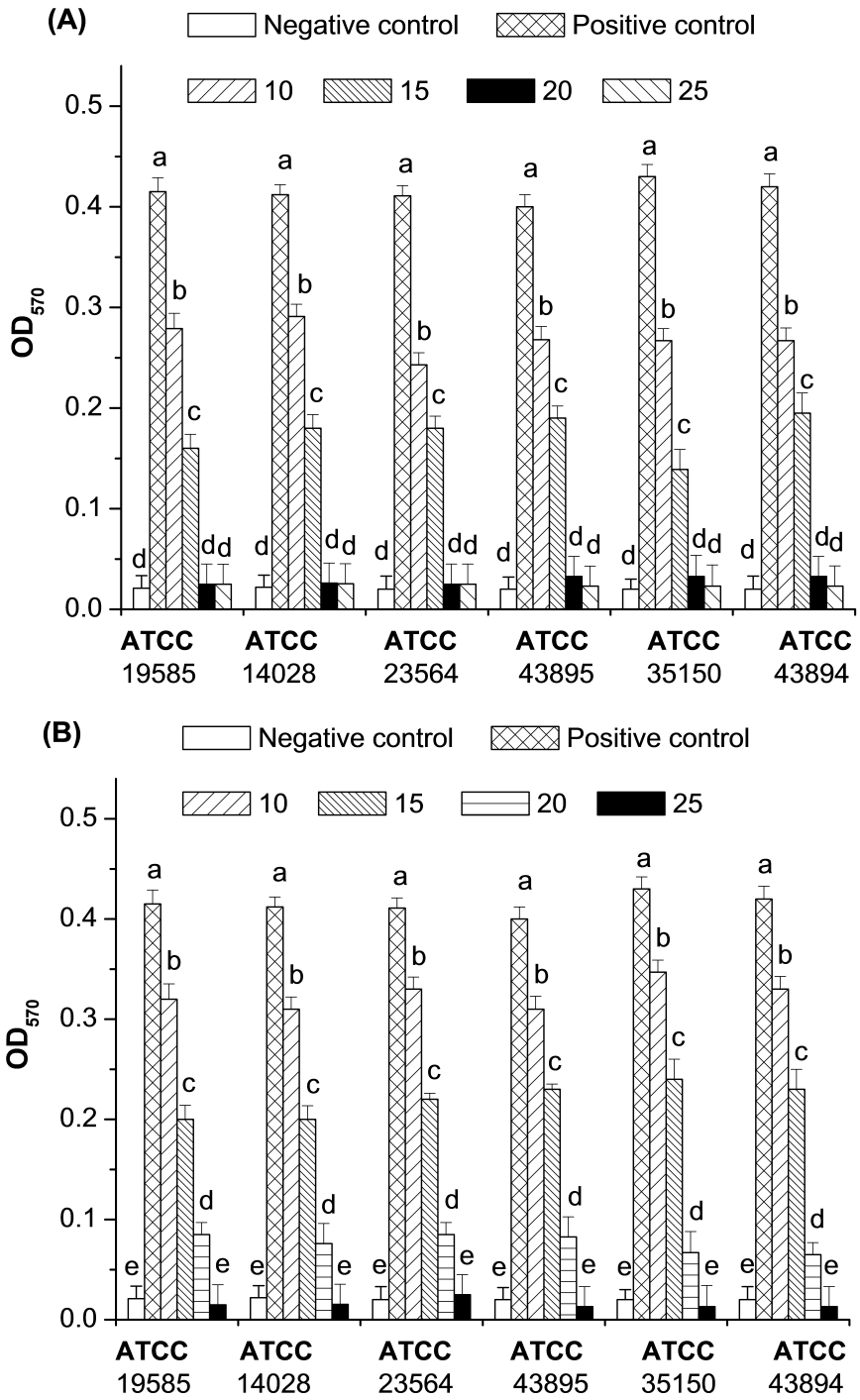

Fig 2: Anti-adhesive effect of (A) fresh (FGE) and (B) cooked (CGE) ginger water extracts against *S.* Typhimurium strains ATCC 19585, ATCC 14028 and ATCC 23564 and *E. coli* O157:H7 strains ATCC 43895, ATCC 35150 and ATCC 43894. Tested concentrations were prepared by dissolving per 1 mL: 0, 10, 15, 20, 25 or 30 mg of the dried total soluble solids (DTSS) of FGE and CGE. Values are means ±SD, $n = 3$. Columns with the same letter within each group are insignificantly different ($P>0.05$).

Table 3: Anti-adhesive effect of ginger water extracts against *S.* Typhimurium strains and *E. coli* O157:H7.

Strains	Number of adhered bacteria per HEp2 cells*				
	Control	FGE	% reduction	CGE	% reduction
S. Typhimurium					
ATCC 19585	51 ±4	32 ±3	37.3	38 ±3	25.5
ATCC 14028	43 ±6	22 ±3	51.2	33 ±2	23.3
ATCC 23564	54 ±4	29 ±4	46.3	33 ±2	38.9
Escherichia coli O157:H7					
ATCC 43895	50 ±6	34 ±4	32.0	35 ±3	30.0
ATCC 35150	52 ±4	23 ±3	55.8	33 ±4	36.5
ATCC 43894	52 ±3	37 ±4	28.8	41 ±4	21.2

**The mean number (n = 3) of bacteria ± SD per HEp-2 cell after examination of at least 100 different cells from five separate fields.*
[a]Strains were classified as no biofilm producer (-), weak biofilm producer (+), moderate biofilm producer (++) or strong biofilm producer (+++).
[b]DTSS is the dried total soluble solids expressed per 1 g fresh ginger rhizome (w/w).
[c]FGE and CGE are fresh and cooked ginger water-extract respectively; which were prepared by dissolving per 1 mL: 0, 10, 15, 20, or 25 mg DTSS.

The minimum anti-adhesive concentration (MAAC; that caused complete prevention of cell adhesion) of FGE and CGE was 20 and 25 mg DTSS/ mL, respectively (Fig 2). In other words, FGE had strong effect as anti-adhesive agent against *S.* Typhimurium strains and *E. coli* O157:H7 compared with the CGE. This may due to the high content of gingerols in FGE (Table 1). Biofilm forming ability of *S.* Typhimurium strains and *E. coli* O157:H7 was also studied (Table 2). All studied strains showed strong ability to form biofilm after 24h in the absence of ginger extracts, except *S.* Typhimurium ATCC 14028 (Table 2). In harmony with this result, strain ATCC 14028 has been described previously as moderate biofilm producer compared with the other *Salmonella* strains (Soni *et al.,* 2012). In accordance with the results of MAAC, the minimum antibiofilm concentration (MABC) of FGE and CGE were 20 and 25 mg DTSS/ mL, respectively (Table 2). The FGE and CGE prepared in the present study did not showed complete inhibition of bacterial growth, although, it has considerable anti-adhesive and antibiofilm effects. These effects were obtained when both tested extracts have been tested at sub-lethal concentrations. This means that the effect of ginger extracts was due to attenuate adhesion of cells to substratum but not referred to suppression of bacterial growth.

3.4 Anti-adhesive Effect (on HEp-2 cells) of Ginger Water Extract

The anti-adhesive effect of FGE and CGE on *S.* Typhimurium strains and *E. coli* O157:H7 has been studied using cell lines of human origin in culture as *in vitro* models for intestinal epithelium (HEp-2 cells). This experiment was carried out in the absence and presence of the anti-adhesive dose, which has been recorded against each bacterial group (*S.* Typhimurium strains and *E. coli* O157:H7). In the absence of ginger extracts, the adhered bacterial cells were ranged from 43 ±6 to 54 ±4 cells per HEp-2 cell, respectively. The anti-adhesive activity of ginger extracts was expressed as a reduction percentage of adhesion and presented in Table 3. The highest inhibition percentage of bacterial cell adherence to HEp-2 was recorded by strain 14028, which has been characterized by its low ability to form biofilm (Table 3). In general, anti-adhesive activity of FGE and CGE was ranged from 28.8 to 55.8% and from 21.2 to 38.9%, respectively. Kim and Park (2013) have reported that ginger extract has ability to inhibit *Pseudomonas aeruginosa* PA14 biofilm formation, where biofilm development was reduced by 39–56% in a static biofilm assay using microtiter plates. The obtained results demonstrated the anti-adhesive potential of the FGE compared with that of CGE. This may be due to the high content of 6-, 8-, 10-gingerls as bioactive components in FGE compared with that of CGE. In this respect, 6-gingerol of fresh ginger has reduced biofilm formation and virulence by antagonistically binding to *P. aeruginosa* quorum sensing (QS) receptors (Kim *et al.,* 2015).

4. Conclusion

The obtained results have strongly indicated that the fresh and cold ginger extracts prepared in the present study exhibited a partial antimicrobial activity and a significant anti-adhesive effect against the Gram-

negative *S.* Typhimurium strains and *E. coli* O157:H7. However, the fresh water extract of ginger displayed anti-adhesive and antibiofilm potential higher than those observed by the cooked ginger extract.

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