

Molecular Effects and Antibacterial Activities of Ginger Extracts against Some Drug Resistant Pathogenic Bacteria

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IN THIS study, the antibacterial activity of six types of ginger extracts was evaluated against drug resistant *E. coli*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. The impact of ginger methanol extract on the tested bacteria was studied at the molecular level via SDS-PAGE and RAPD-PCR methods. From the six types of ginger extracts, ginger methanol extract recorded a remarkable antimicrobial activity and the minimum inhibitory concentration was 0.7 mg/ml against all tested bacteria. The Gram positive bacteria showed more sensitivity to ginger methanol extract and lower growth rate pattern than the Gram negative bacteria. The qualitative phytochemical analysis of ginger methanol extract recorded the presence of steroids and flavonoids that are wide-range antimicrobial agents. From GC-MS results, sixteen compounds were identified with major compounds gingerol (30.56%), cis-6-shagol (21.61%), zingerone (8.22%) and 2,4-dimethyl- Benzo[h]quinolone (5.48%). The combination of ginger methanol extract with gentamycin or amoxicillin antibiotics improved its antibacterial activity. The biomarker assay detecting the protein changes based on SDS-PAGE profile and the genetic changes based on RAPD-PCR manipulation of ginger methanol extract manifested a polymorphic pattern when treated and untreated bacteria were compared. In conclusion, the application of ginger methanol extract increased the activity of gentamycin and amoxicillin antibiotics and because ginger methanol extract has effective and safe bioactive antimicrobial agent against Gram positive and Gram negative bacteria, it can be used as an alternative drug.

Keywords: GC-MS, Ginger extracts, Bacteria, Protein, RAPD-PCR.

Introduction

Searching for new antibacterial agents from natural sources was enforced by the emergence of antibiotic resistant bacteria due to the indiscriminate use of antibiotics (Abiramasundari et al., 2011). Medicinal plants might be in that focus because they are rich with gingerols, Zingerone, shogaols and other bioactive compounds which belong to different classes such as tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Yadav & Agarwala, 2011).

Since long time, ginger (*Zingiber officinale*) is one of largely used spices which has been used in conventional oriental medicines as anticancer (Wang et al., 2002 and Wei et al., 2005), anti-inflammatory (Habib et al., 2008), antimicrobial and antifungal agent (Park et al., 2008). Fresh ginger has been used for treatment of nausea, cold-induced disease, colic, asthma, cough,

heart palpitation, swellings, dyspepsia, loss of appetency, and rheumatism (Wichtl, 2004). *Z. officinale* is an herbaceous perennial plant belonging to the family Zingiberaceae and has phenolic compounds such as gingerols (gingerol-related compounds), shogaols, sesquiterpenes and zingiberenes. The major pungent components of ginger are 6-gingerol and 6-paradol. Ginger rhizome also contains diarylheptanoids which have different shapes such as a linear shaped curcumin and a cyclic shaped myricanone (Park et al., 2008; Nazari et al., 2015 and Arshad & Shadab, 2017).

Ginger extracts have different degrees of antibacterial potentiality (Wei et al., 2005 and Arshad & Shadab, 2017), where the essential oil of ginger was stronger than the oleoresin against *E. coli* and *S. aureus* (Bellik, 2014). Ginger aqueous extract has weak effect on *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus*

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epidermidis, *S. aureus*, *Klebsiella pneumoniae*, *Shigella sonnei*, and *Salmonella typhi* (Gull et al., 2012), where as the antimicrobial effects of ginger extract in peptone water buffer against *E. coli* O157:H7 was strong (Gupta & Ravishankar, 2005). On the other hand, some bacterial strains such as *Salmonella enterica* serotype, *Typhimurium* and *E. coli* O157: H7 were resistant to ethanol ginger extract (Pattaratanawadee et al., 2006).

The huge use of antibiotics have developed multiple drug resistance (MDR) of many pathogenic bacteria (Pattaratanawadee et al., 2006). This study aimed to investigate the antibacterial activity of ginger extracts and identify the compounds of the most active ginger extract (ginger methanol extract) by GC-MS analysis, and also to evaluate the synergistic effect of ginger methanol extract with amoxicillin and gentamicin antibiotics. In addition, the impact of ginger methanol extract on the four tested pathogenic bacteria (*E. coli*, *Shigella dysenteriae*, *Staphylococcus aureus* and *S. epidermidis*) was performed at the molecular level.

Materials and Methods

Plant material

Ginger rhizome powder was purchased from local Egyptian market and stored in dry condition at room temperature.

Tested bacteria

Four different characterized drug resistant bacterial strains including *E. coli* and *S. dysenteriae* (Gram negative), *S. aureus* and *S. epidermidis* (Gram positive), were used in this study. The strains were preserved on LB agar medium at 4°C and were sub-cultured at 37°C for 24 h every month. These organisms were originally obtained from the culture collection of Department of Microbiology and Immunology, Faculty of Medicine, Mansoura University, Egypt.

Preparation of ginger extracts

The method of Gull et al. (2012) was used for the preparation of ginger extracts. The ginger powder (10 g) was extracted with 100 ml of each organic solvent (water, methanol, ethanol, acetone, chloroform and petroleum ether) separately for 3 days at room temperature. All types of extracts were filtered with filter papers and then dried in oven at 40°C except water extract which was dried at 80°C. Oven-dry extracts were dissolved in 10% Dimethyl sulphoxide (DMSO) with a final concentration 3 mg/ml.

Antibacterial bioassay of ginger extracts using disc diffusion method

To evaluate the antibacterial activity of the six types of ginger extracts, the disc agar diffusion method was used as described by Bhargava et al. (2012). LB agar plates were inoculated separately with 10^7 CFU of every bacterial strain culture and regularly spread on whole surface of each plate. The 5 mm diameter sterile discs were saturated with 10 µl of the different extracts and placed on LB plates inoculated with bacterial culture. The plates were incubated for 24 h at 37°C and observed for zone of inhibition. The diameter of inhibition zones were measured in millimeters. 10% DMSO was used as negative controls and standard reference antibiotics; gentamycin (10 µg/disc) and amoxicillin (30 µg/disc) were used as positive controls for the tested bacteria. Each assay in this test was done in three replicates.

Statistical analysis

Each experiment was run in triplicate, and mean values were calculated. The statistical analysis was carried out employing one way ANOVA ($p < 0.01$). A statistical package (SPSS version 16.0) was used for data analysis.

Determination of MIC of ginger methanol extract (GiM)

MIC of ginger extract was determined using disc agar diffusion method as described by Bhargava et al. (2012). Serial dilutions of ginger methanol extract were prepared to obtain concentrations of 0.35, 0.7, 1.4 and 2.8 mg/ml and tested for MIC against bacterial strains. Sterile filter paper discs were saturated with 10 µl of the different dilutions of ginger extract and placed on LB agar plates inoculated with 10^7 CFU of bacterial cultures separately. Plates were placed at 37°C for 24 h. The zone of inhibition in each case was measured as the diameter of the clearing zones and the results were documented. Each test was performed in three replicates.

Phytochemical and GC-MS analysis of ginger methanol extract

Qualitative phytochemical screening was carried out on GiM. The extract was tested for the presence of bioactive compounds such as terpenoids, glycosides, flavonoids, coumarins, alkaloids compounds, tannins, and saponins by using standard methods of Yadav & Agarwala (2011). GiM extract sample was injected in Agilent GC-MS equipment under certain conditions. Wiley and Wiley Nist mass spectral data base was

used in the identification of the spectral peaks, GC-MS was carried out at Central Agricultural Pesticide Laboratory (CAPL), Giza, Egypt.

Influence of ginger methanol extract on bacterial growth

Turbidity method

Influence of GiM extract on the tested bacteria was evaluated by culturing in LB broth media containing the measured MIC value for bacterial strains (0.7 mg/ml) as a final concentration. LB broth media were inoculated with inoculum size of 10^6 CFU/ml of each bacterial strain and the inoculated media was incubated in the incubation shaker (37°C, 150 rpm). The growth of bacteria was determined by measuring the optical density of the culture at 600 nm every 2 h and up to 6 h using UV-visible spectrophotometer. The culture without extract was used as control (Elazomi et al., 2016).

Viable count method

For viable count method, each of the tubes inoculating with bacterial suspension (approximately 5.0×10^4 CFU/ml) of each bacterial strain in LB broth medium was inoculated with MIC concentration in 50 ml LB broth, and kept at 37°C for overnight. The cultures were diluted several times (10^{-1} , 10^{-2} , 10^{-3} , and 10^{-4}) in LB broth media and 100 µl of each dilution was inoculated on LB agar plate. The viable count was recorded as colony forming units per ml (CFU/ml), after incubation at 37°C for 24 h. The controls were inoculated without ginger extract for each bacterial strain with the same conditions as mentioned above. For counting, only plates that contained a number of colonies ranging from 30 to 300 were selected (Lawal et al., 2015).

Synergistic effect of GiM extract with antibiotics

Single impact of GiM extract, amoxicillin (AX) and gentamicin (GM) as well as combinations of AX+GiM and GM+GiM were performed. Commercially antibacterial AX discs (30 µg) and GM discs (10 µg) were saturated with 10 µl GiM extract (0.7 mg/ml) under aseptic conditions and then were applied on the surface of LB agar media freshly inoculated by the tested bacteria. The plates were incubated at 4°C for 20 min and then were transferred to 37°C for 24 h. After the incubation period, the diameters of the inhibition zones formed were measured in mm and then compared with each other (Hudzicki, 2009).

SDS-PAGE

The four tested bacteria treated with GiM and

untreated (control) were cultured in LB broth media at 37°C for 24 h. The bacterial cells were harvested by centrifugation at 10,000 rpm for 5 min. The pellets were homogenized in phosphate buffer (0.6 M, pH 6.8) using glass beads and FastPrep®-24 homogenizer and then centrifuged at 10,000 rpm for 5 min. Ten µl protein samples from each bacteria were boiled with 30 µl of 2X sample buffer (10 ml Distilled Water, 2.5 ml Tris-HCl pH 6.8, 2 ml Glycerol, 4 ml of 10% SDS and 1 ml β-mercaptoethanol) for 2 min, cooled immediately on ice and 20 µl of lysed cell product were loaded over acrylamide gel. Acrylamide gel was prepared according to Laemmli (1970) from two layers; 4% stacking gel on top of 12% separating gel. After electrophoresis at 100 V for 2 h, gel was overnight stained in Commassie brilliant blue R250 and visualized by soaking in destaining solution on shaker for some hours. The gel was documented and analysed using gel analyser 3 programme.

RAPD-PCR

The four tested bacteria were cultured in LB broth media provided with the MIC of GiM extract at 37°C for overnight. The genomic DNA was isolated from the bacterial pellets according to the instructions of GeneJET Genomic DNA purification Kit (Thermo scientific, Germany). The purified DNA was used as a template RAPD-PCR reaction using three primers (U16-25-5-CTGCGCTGGA3', T16-25-5-GGTGAACGCT3' and K02-25-5-GTCTCCGCAA3'). The reaction mixture was adjusted with a total volume of 20 µl: 1 µl DNA template, 4 µl 5x master-mix buffer, 2 µl primer, 0.5 µl Taq DNA polymerase and 12.5 µl distillate water. The PCR program was: 94°C for 3 min, 94°C for 1 min, 30°C for 30 sec, 72°C for 1 min, 72°C for 5 min (40 cycles). The PCR products were detected on 1.2% agarose gel by gel documentation system (Nippon Genetics Company), followed by introducing to Gel Analyser program 3 for analysis.

Results

The antibacterial activity of ginger extracts

The antimicrobial activity of the six types of ginger extracts were measured as inhibition zones (mm) against the four tested pathogenic bacteria (Fig. 1). The results indicated that different ginger extracts had a widerange antibacterial activity with different degrees of sensitivity of tested pathogenic bacteria. Growth inhibition was not observed around the control disc containing

DMSO. Fig. 1 shows that ginger water extract (GiW), ginger ethanol extract (GiE) and ginger petroleum ether extract (GiP) had no effect on *S. dysenteriae*, *E. coli* and *S. aureus*, respectively. On the other hand various extracts of ginger affected the growth of the tested pathogenic bacteria with variable degrees of inhibition zones. Ginger methanol extract (GiM), ginger acetone extract (GiA) and ginger chloroform extract (GiC) showed variable inhibition zones ranging from (6-12 mm) on all tested pathogenic bacteria. The results also showed that Gram positive and Gram negative bacteria were sensitive to most of the extracts of ginger, however Gram positive bacteria were more sensitive than Gram negative bacteria. *S. epidermidis* was affected by the six types of ginger extracts more than the other tested bacteria. GiM extract recorded wide broad spectrum antimicrobial activity. Therefore ginger methanol extract was selected for further studies.

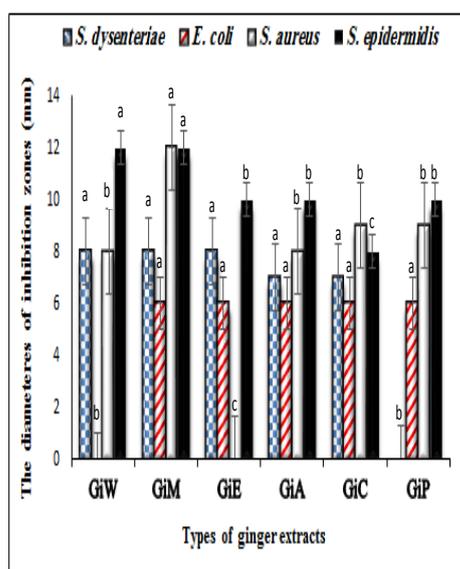


Fig. 1. Mean of inhibition zone (mm) of 6 types of medicinal plant extracts from ginger against four drug resistant pathogenic bacteria *S. dysenteriae*, *E. coli*, *S. aureus* and *S. epidermidis* using disc agar diffusion method.

(GiW; ginger water extract, GiM; ginger methanol extract, GiE; ginger ethanol extract, GiA; ginger acetone extract, GiC; ginger chloroform extract, GiP; ginger petroleum ether extract); Data are the means of three replicates and errors bars represent the standard errors of the means. Means within the same bacteria denoted with different letters are significantly different at $p < 0.01$

The MIC of GiM extract

The results showed that the MIC of ginger methanol extract (GiM) was 0.7 mg/ml with all of the tested bacteria.

Phytochemical screening and GC-MS analysis of GiM extract

The qualitative phytochemical analysis of methanol extract of ginger showed the presence of triterpenes, carbohydrates, flavonoids, alkaloid compounds, tannins and saponins. Table 1 showed the GC-MS profile of GiM extract and the relative amount of each compound. Sixteen compounds from GiM extract were identified with major substances gingerol (30.56%), cis-6-shagol (21.61%), zingerone (8.22%) and 2,4-dimethyl-Benzo[h]quinolone (5.48%).

Effect of GiM extract on the growth of the tested bacteria

Growth curve analysis

The comparison of the growth patterns of treated bacteria with GiM and untreated (Fig. 2) showed that, the growth of the four untreated bacteria increased with different rates during the whole incubation period. The rate of growth of *S. aureus* and *S. dysenteriae* in absence of GiM extract was better than *E. coli* and *S. epidermidis*. On the other hand, the growth of all bacteria was inhibited by GiM treatment. Gram positive bacteria *S. aureus* and *S. epidermidis* were more sensitive to GiM extract than Gram negative bacteria *S. dysenteriae* and *E. coli*.

Bacterial viable count analysis

The viable count of the four tested bacteria recorded reduction of differed degrees under the effect of the MIC of GiM extract (Table 2). The maximum reduction 86.8% appeared with *S. epidermidis*, while the minimum reduction 51.7% appeared with *S. dysenteriae*. In between, *E. coli* recorded growth inhibition 66.1% and *S. aureus* recorded growth inhibition 79.6%.

The synergistic effect of GiM extract with antibiotics

Single impact of GiM extract showed a maximum inhibition zone (12 mm) on *S. aureus*. While, both amoxicillin (AX) and gentamycin (GM) separately recorded a maximum inhibition zone of 18 and 27 mm, respectively against *E. coli*. Combination of GiM extract and AX maximized the activity of AX by 22.2% against *S. epidermidis*. Also combination of GiM extract with GM maximized the activity of inhibition of GM by 27.6% against *S. dysenteriae* (Fig. 3).

TABLE 1. GC-MS analytical results of ginger methanol extract.

No	Retention time (min)	Compounds	Area (%)
1	6.196	Isometric dihydro-methyl-furanone	1.57
2	11.605	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	3.94
3	16.228	2-Methoxy-4-vinylphenol	1.30
4	18.423	4-hydroxy-3-methoxy Benzaldehyde	4.54
5	24.397	Zingerone	8.22
6	27.873	β -Oplophenone	4.78
7	28.059	1-Ethylideneoctahydro-7a-methyl-1H-iodene	3.01
8	29.270	Cyclohexanecane	3.99
9	35.686	6-Amino-2,4-dimethylphenol	0.77
10	36.874	Cis-6-shogaol	21.61
11	38.475	Gingerol	30.56
12	39.383	Octadecanoic acid	1.68
13	40.245	Capsaicin	2.26
14	40.973	Benzene di-carboxylic acid	3.87
15	43.418	2-[4-Chlorophynyl]-5-pyrimidinamin;	2.42
16	50.719	2,4-dimethyl- Benzo[h]quinolone	5.48

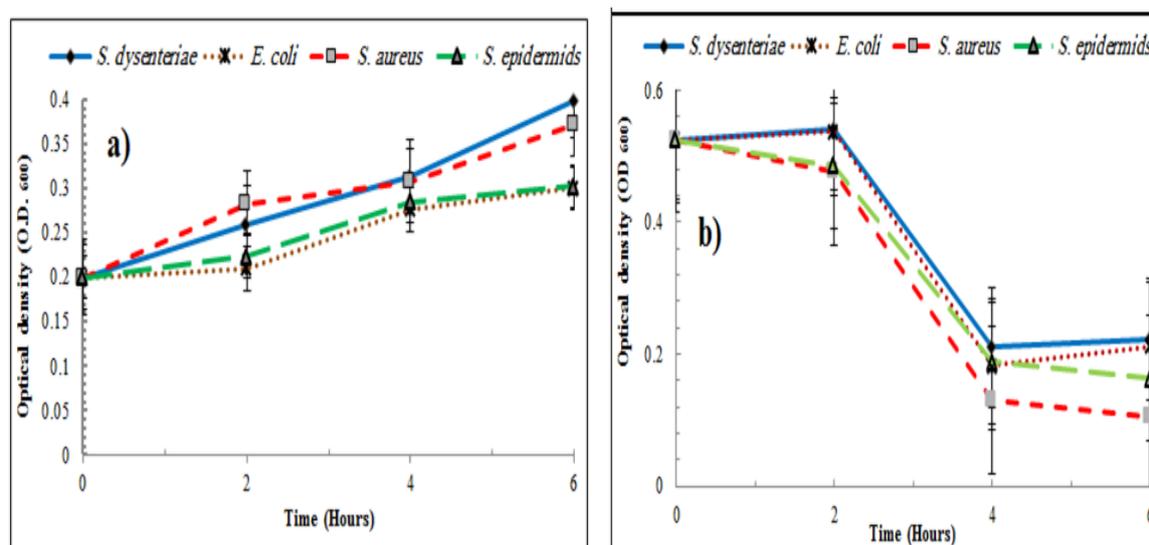


Fig. 2. The growth curve (OD 600); a) Untreated bacteria (control) and b) Treated bacteria (by MIC value of GiM). *S. dysenteriae*, *E. coli*, *S. aureus* and *S. epidermidis* bacterial cultured in LB broth media. Data are the means of three replicates and errors bars represent the standard errors of the means.

TABLE 2. Viable count and percent of bacterial growth inhibition of pathogenic bacteria untreated (control) and treated with the MIC value of ginger methanol extract.

Types of bacteria	CFU/ml			Bacterial growth inhibition (%)
	0.0 h	Untreated, 24 h	Treated, 24 h	
<i>S. dysenteriae</i>	5.0×10^4	5.8×10^8	2.8×10^8	51.7%
<i>E. coli</i>	5.1×10^4	5.9×10^8	2.0×10^8	66.1%
<i>S. aureus</i>	5.6×10^4	6.4×10^8	1.3×10^8	79.6%
<i>S. epidermidis</i>	5.3×10^4	6.1×10^8	1.4×10^8	86.8%

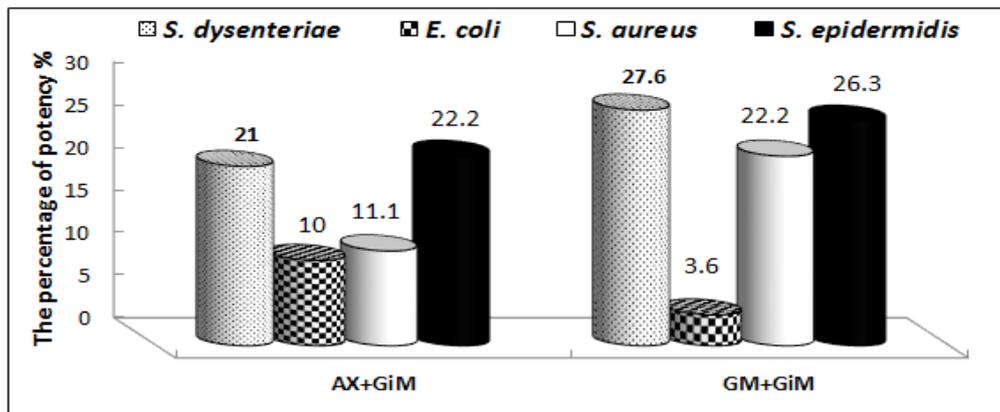


Fig. 3. The increasing of antibiotic activity of combination AX+GiM and GM+GiM against *S. dysenteriae*, *E. coli*, *S. aureus* and *S. epidermidis* as expressed by the increasing in inhibition zone diameter (AX+GiM; amoxicillin + ginger methanol extract; GM+GiM; gentamycin + ginger methanol extract).

Effect of GiM extract on the protein pattern of the tested bacteria

Protein profile of the four tested bacteria treated with GiM extract was documented in Fig. 4 and Table 3. In case of *S. dysenteriae*, the total protein bands were 40, distributed as 34 monomorphic and 6 polymorphic bands. In case of *E. coli*, the total bands were 36 bands, distributed as 32 monomorphic bands and polymorphic

bands. In case of *S. aureus*, the total bands were 38 bands distributed as 28 monomorphic and 10 polymorphic bands. In case of *S. epidermidis*, the total bands were 40 bands distributed as; 38 monomorphic and 2 polymorphic bands. The highest percentage of polymorphism was obtained with *S. aureus* (26.3%) and the lowest percentage of polymorphism was obtained with *S. epidermidis* (5.0 %).

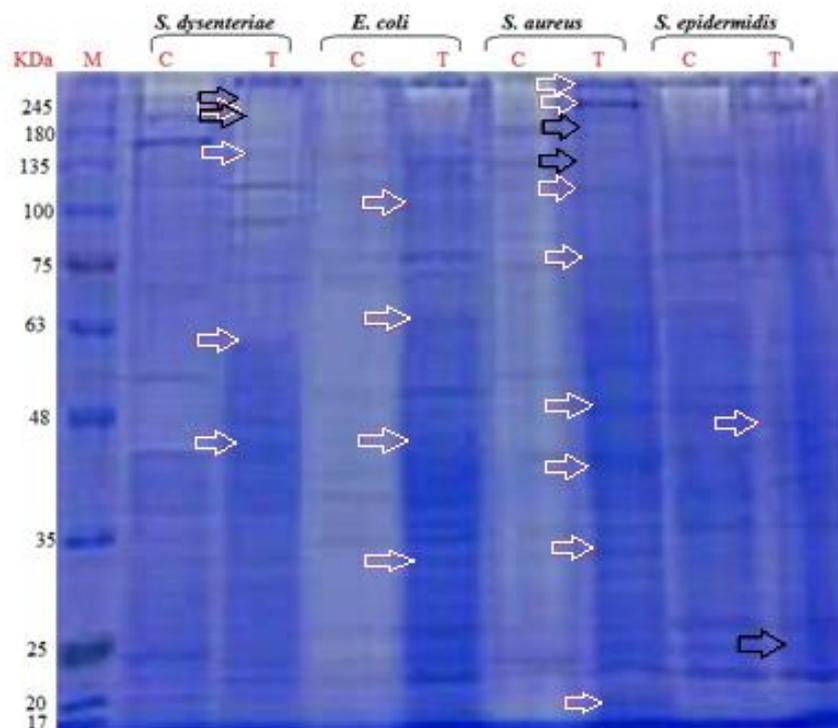


Fig. 4. Protein pattern of *S. dysenteriae*, *E. coli*, *S. aureus* and *S. epidermidis* bacteria cultivated on LB broth media provided with GiM extract; KDa= Kilo Dalton, M= marker, C= control; untreated bacteria, T= treated bacteria with GiM extract, black arrow= disappeared band, white arrow= new band.

TABLE 3. Comparative analysis of relative protein band percentages of *S. dysenteriae*, *E. coli*, *S. aureus* and *S. epidermidis* bacteria, treated with the MIC of ginger methanol extract for 24 h and untreated bacteria by using SDS-PAGE technique.

No. of Bands	Bacteria			
	<i>S. dysenteriae</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>S. epidermidis</i>
Control	19	16	16	21
Treated	21	20	22	19
Monomorphic No. (%)	34 (85.0)	32 (89.0)	28 (73.7)	38 (95.0)
Polymorphic No. (%)	6 (15.0)	4 (11.0)	10 (26.3)	2 (5.0)
Total No.	40	36	38	40

Effect of GiM extract on DNA of pathogenic bacteria

The stability of the bacterial genomic DNA after the treatment with the GiM extract was evaluated with using RAPD analysis. The RAPD results illustrated in Table 4 and Fig. 5., show polymorphic numbers and percentage of the genetic bands, which were the electrophoretic yields of PCR for treated bacteria compared with those of untreated bacteria. Table 4 demonstrates that highest total number of polymorphic bands

among treated *S. dysenteriae* was obtained in reactions with primers T16-25 and K02-25 which were 10 and 6, respectively of genetic bands and represented 83.3 and 100%, respectively of total bands. While, the highest number among treated *E. coli* was obtained in reactions with primers U16-25 and K02-25 which was 5 and 4 genetic bands, respectively and represented 45.5 and 100%, respectively of total bands. But, the highest number among *S. aureus* and *S. epidermidis* was obtained with all primers.

TABLE 4. Comparative analysis of relative DNA band percentages of *S. dysenteriae*, *E. coli*, *S. aureus* and *S. epidermidis* bacteria, treated with the MIC of GiM extract for 24 h and untreated bacteria by using three DNA-primers; a) U16-25, b) T16-25 and c) K02-25.

No. of bands	Bacteria											
	<i>S. dysenteriae</i>			<i>E. coli</i>			<i>S. aureus</i>			<i>S. epidermidis</i>		
Primers	a	b	c	a	b	c	a	b	c	a	b	c
Control	5	8	2	6	3	2	5	4	4	2	4	4
Treated	5	4	4	5	3	2	3	3	1	6	2	2
Monomorphic No. (%)	10(100)	2(16.6)	0(0.0)	6(54.5)	6(100)	0(0.0)	4(50.0)	4(57.2)	0(0.0)	2(25.0)	0(0.0)	0(0.0)
Polymorphic No. (%)	0(0.0)	10(83.3)	6(100)	5(45.5)	0(0.0)	4(100)	4(50.0)	3(42.8)	5(100)	6(57.0)	6(100)	6(100)
Total No.	10	12	6	11	6	4	8	7	5	8	6	6

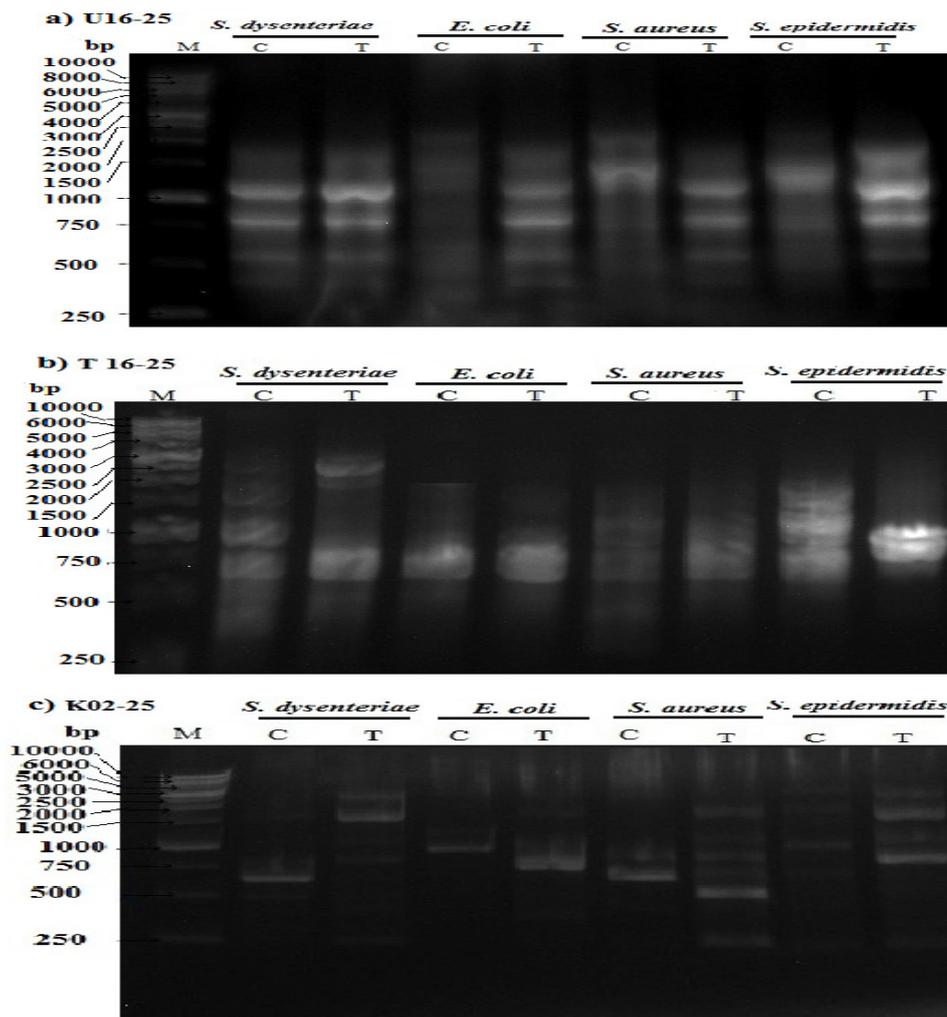


Fig. 5. RPAD-PCR of tested bacteria using three DNA-primers; a) U16-25, b) T16-25 and c) K02-25 RAPD-primers. (M): 1 Kb DNA ladder. bp= base pair, M=marker, C= control, T= treated with ginger methanol extract.

Discussion

Treatment with antibiotics is not only expensive, but the risk of bacterial resistance to antimicrobial agents and side effects such as acidity burning sensation and damage to natural fauna of intestine are also involved. In this study, from the six types of ginger extracts only GiM extract recorded the highest antimicrobial activity with MIC (0.7 mg/ml) against *S. dysenteriae*, *E. coli*, *S. aureus* and *S. epidermidis* where Gram positive bacteria were more affected than Gram negative bacteria. Arshad & Shadab (2017) reported that methanol extract has better potential than hexane. Generally, different plant extracts differ in their anti-bacterial activities against different bacteria due to presence of different bioactive compounds. Also, different ginger extracts (distilled water, acetone, methanol and ethanol) have an antibacterial activity on *S. aureus*

(Noor et al., 2011). This matched with Kaushik & Goyal (2011) who compared among water, ethanol, methanol, hexane and ethyl acetate ginger extracts against *E. coli* and *S. aureus*. This might be due to that GiM extract has different chemical constituents and secondary metabolites (Ghareeb et al., 2014). On the other hand, Sundar et al. (2015) found ethanol extract inhibited the growth of *E. coli* more than methanol extract. Also, ginger ethanol and methanol extracts were more active against *Shigella* spp., *S. epidermidis*, *E. coli* and *S. aureus* than ginger aqueous extracts (Gull et al., 2012). Depend on solubility of the active constituents, different extracts of spices or herbs differ in their anti-microbial activities against different bacteria and this might be due to the presence of different active phyto-compounds (Das, 2012).

Gram positive bacteria *S. aureus* and *S.*

epidermidis showed more sensitivity to GiM extract besides lower growth rate pattern as compared with Gram negative bacteria *S. dysenteriae* and *E. coli*. The differences in growth pattern between Gram positive and Gram negative might be attributed to the dissimilarity of cell wall composition or the respiration pathway or the presence of some genes on the bacterial chromosome or plasmid that might be able to inactivate or degrade the active compounds in the GiM extract (Yang et al., 2000). Gram negative bacteria have an efficient permeability barrier, included of outer membrane, which limits the penetration of amphipathic compounds and multidrug resistance pumps that extrude toxins across this barrier. It is possible that the seeming ineffectiveness of plant antimicrobials is largely due to the permeability barrier (Tegos et al., 2002). Phytochemical analysis of GiM extract contained terpenoids, glycosides, flavonoids, alkaloids compounds, tannins, and saponins (Yadav & Agarwala, 2011). Steroids are antibacterial compounds (Raquel, 2007), flavonoids, wide range antimicrobial agents, able to complex with bacterial cell wall (Cowan, 1999). The major compounds in ginger constitutes were gingerol, cis-6-shagol and zingerone and these matched with Arshad & Shadab (2017).

The combination of GiM extract improved the efficiency of the gentamycin antibiotic more than the combination with the amoxicillin antibiotic, which is going parallel with Aburjai et al. (2001). In another study, the combination of methanol ginger extract and tetracycline antibiotic against *S. aureus* was synergistic (Betoni et al., 2006). Also, Jouda et al. (2015) found synergistic effect between methanol extracts of *Artemisia herbaalba*, *Lantana camara*, *Allium sativum* and *Eucalyptus camaldulensis* and gentamicin against *S. aureus* and *E. coli*. In previous researches, a synergism of various plant extracts and antibiotics against some pathogenic bacteria was noticed (Betoni et al., 2006 and Shaaban et al., 2013).

The biomarker assay detecting the protein changes based on SDS-PAGE profile and the genetic changes based on RAPD-PCR manipulation of GiM extract manifested a polymorphic pattern when comparing between the treated and untreated bacteria. This genetic changes represented in disappearance of some bands verify the capacity of GiM extract compounds such as tannins and alkaloids to generate some kind of mutation or genetic disorder or at least

one nucleotide change (point mutation) which disturb the gene expression as well as the DNA and protein synthesis (Owen & Johns, 1999 and Gilani et al., 2006). In conclusion, GiM extract has effective and safe bioactive antimicrobial agents against Gram negative and Gram positive bacteria. Also GiM extract increased the activity of gentamycin and amoxicillin antibiotics.

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References

- Abiramasundari, P., Priya, V., Jeyanthi, G.P. and Gayathri, D. (2011) Evaluation of the antibacterial activity of *Cocculus hirsutus*. *Journal Drugs Medicines*, **3**, 26-31.
- Aburjai, T., Darwish, R.M., Al-Khalil, S., Mahafzah, A. and Al-Abbadi, A. (2001) Screening of antibiotic resistant inhibitors from local plant materials against two different strains of *Pseudomonas aeruginosa*. *Journal of Ethnopharmacology*, **76**(1), 39-44.
- Arshad, M. and Shadab, M. (2017) *Zingiber officinale* extract: Antimicrobial properties phytochemical screening, drug likeness and physicochemical studies. *European Journal of Pharmaceutical and Medical Research*, **4**(3), 364-368.
- Bellik, Y. (2014) Total antioxidant activity and antimicrobial potency of the essential oil and oleoresin of *Zingiber officinale* Roscoe. *Asian Pacific Journal of Tropical Disease*, **4**(1), 40-44.
- Betoni, J.E.C., Mantovani, R.P., Barbosa, L.N., Di Stasi, L.C. and Fernandes Junior, A. (2006) Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. *Memorias do Instituto Oswaldo Cruz*, **101**(4), 387-390.
- Bhargava, S., Dhabhai, K., Batra, A., Sharma, A. and Malhotra, B. (2012) *Zingiber officinale*: Chemical and phytochemical screening and evaluation of its antimicrobial activities. *Journal of Chemical and Pharmaceutical Research*, **4**(1), 360-364.
- Cowan, M.M. (1999) Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, **12**(4), 564-582.
- Das, S., Anjeza, C. and Mandal, S. (2012) Synergistic or additive antimicrobial activities of Indian spice and herbal extracts against pathogenic, probiotic and food-spoiler micro-organisms. *International Food Research Journal*, **19**(5), 1185-1191.

- Elazomi, A., Rahman, M.E., Liddell, S., Lovell, M. and Barrow, P. (2016) Determination of the proteins of *Salmonella enteritidis* involved in colonization of the chicken ceca using protein analysis. *Imperial Journal of Interdisciplinary Research*, **2**(5), 1729-1738.
- Ghareeb, M.A., Shoeb, H.A., Madkou, H.M.F., Refaey, L.A., Mohamed, M.A. and Saad, A.M. (2014) Antioxidant and cytotoxic activity of *Tectona grandis* Linn leaves. *International Journal of Phytopharmacology*, **5**(2), 143-157.
- Gilani, S.A., Kikuchi, A., Shinwari, Z.K., Khattak, Z.I. and Watanab, K.N. (2006) Phytochemical, pharmacological and ethanotanical studies of *Rhazya stricta* Decne. *Phytotherapy Research*, **21**, 301-307.
- Gull, I., Saeed, M., Shaukat, H., Aslam, S.M., Samra, Z.Q. and Athar, A.M. (2012) Inhibitory effect of *Allium sativum* and *Zingiber officinale* extracts on clinically important drug resistant pathogenic bacteria. *Annals of Clinical Microbiology and Antimicrobials*, **11** (1), 8-11
- Gupta, S. and Ravishankar, S. (2005) A comparison of the antimicrobial activity of garlic, ginger, carrot, and turmeric pastes against *Escherichia coli* O157: H7 in laboratory buffer and ground beef. *Foodborne Pathogens and Disease*, **2**(4), 330-340.
- Habib, S.H.M., Makpol, S., Hamid, N.A.A., Das, S., Ngah, W.Z.W. and Yusof, Y.A.M. (2008) Ginger extract (*Zingiber officinale*) has anti-cancer and anti-inflammatory effects on ethionine-induced hepatoma rats. *Clinics*, **63**(6), 807-813.
- Hudzicki, J. (2009) Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiol.* 1-23.
- Jouda, M.M., Elbashiti, T., Masad, A. and Albayoumi, M. (2015) The antibacterial effect of some medicinal plant extracts and their synergistic effect with antibiotics. *Research Article*, **5**(2), 23-33.
- Kaushik, P. and Goyal, P. (2011) Evaluation of various crude extracts of *Zingiber officinale* rhizome for potential antibacterial activity: A study *in vitro*. *Advances in Microbiology*, **1**(1), 7-12.
- Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680-685.
- Lawal, D., Burgess, C., McCabe, E., Whyte, P. and Duffy, G. (2015) Development of a quantitative real time PCR assay to detect and enumerate *Escherichia coli* O157 and O26 serogroups in bovine recto-anal swabs. *Journal of Microbiological Methods*, **114**, 9-15.
- Nazari, A., Fanaei, H., Dehpour, A.R., Hassanzadeh, G., Jafari, M., Salehi, M. and Mohammadi, M. (2015) Chemical composition and hepatoprotective activity of ethanolic root extract of *Taraxacum syriacum* Boiss against acetaminophen intoxication in rats. *Bratislavske Lekarske Listy*, **116**(1), 41-46.
- Noor, A.A., Memon, A.G. and Sherwani, S.K. (2011) Dose response curve of plants extracts against the human pathogens. *Gomal University Journal of Research*, **27**(2), 1-17.
- Owen, P.L. and Johns, T. (1999) Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout. *Journal of Ethnopharmacology*, **64**(2), 149-160.
- Park, M., Bae, J. and Lee, D.S. (2008) Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. *Phytotherapy Research: PTR*, **22**(11), 1446-1449.
- Pattaratanawadee, E., Rachtanapun, C., Wanchaitanawong, P. and Mahakarnchanakul, W. (2006) Antimicrobial activity of spice extracts against pathogenic and spoilage microorganisms. *Kasetsart Journal National Science*, **40**, 159-165.
- Raquel, F.E. (2007) Bacterial lipid composition and antimicrobial efficacy of cationic steroid compounds. *Biochemica et Biophysica Acta*, **4**(1), 2500-2509.
- Shaaban, H., Ahmed, M., Sideek, L. and Amer, M. (2013) Study on the antimicrobial activity and synergistic/antagonistic effect of interactions between antibiotics and some spice essential oils against pathogenic and food-spoiler microorganisms. *Journal of Applied Sciences Research*, **9**(8), 5076-5085.
- Sundar, S., Jayasree, T., Ditya, D., Sanjana, J., Sowmya, M. and Jahnavi, S. (2015) Antimicrobial activity of aminoglycoside antibiotics combined with clove and ginger. *World Journal of Pharmacy and Pharmaceutical Sciences*, **4**(6), 1515-1524.
- Tegos, G., Stermitz, S.R., Lomovskaya, O. and Lewis, K. (2002) Multidrug pump inhibitions uncover remarkable activity of plant antimicrobials. *Antimicrobial Agents Chemotherapy*, **46**(10), 31-33.
- Wang, C.C., Chen, L.G., Lee, L.T. and Yang, L.L. (2002) Effects of 6-gingerol, an antioxidant from ginger, on inducing apoptosis in human leukemic HL-60 cells. *Journal: "In Vivo"*, **17**(6), 641-645.

- Wei, Q.Y., Ma, J.P., Cai, Y.J., Yang, L. and Liu, Z.L. (2005) Cytotoxic and apoptotic activities of diarylheptanoids and gingerol-related compounds from the rhizome of Chinese ginger. *Journal of Ethnopharmacology*, **102**(2), 177-184.
- Wichtl, M. (2004) "*Herbal Drugs and Phytopharmaceuticals: A Handbook for Practice on a Scientific Basis*". 3rd ed. Medpharm Scientific Publisher.
- Yadav, R. and Agarwala, M. (2011) Phytochemical analysis of some medicinal plants. *Journal of Phytology*, **3**(12), 10-14.
- Yang, S., Yu, J. and Xu, L. (2000) Chemical constituents of annonaceae plants and their antitumor activities. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao*. **22**(4), 376-82 <https://www.ncbi.nlm.nih.gov/pubmed/12903455>.

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التأثيرات الجزيئية و الأنشطة ضد بكتيرية لمستخلصات الزنجبيل في بعض البكتريا الممرضة المقاومة للمضادات الحيوية

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في هذه الدراسة تم تقييم النشاط ضد بكتيري لستة أنواع من مستخلصات الزنجبيل ضد أربعة أنواع من البكتريا الممرضة المقاومة للمضادات الحيوية (*S. epidermidis* و *S. aureus* و *E. coli* و *S. dysenteriae*) حيث سجل مستخلص الزنجبيل بالميثانول أفضل نشاط ضد بكتيري وكان أقل تركيز مشط له ٧,٠ ملجم/مللى ضد كل البكتريا المختبرة. و أظهرت البكتريا الموجبة الجرام حساسية أكثر لمستخلص الزنجبيل بالميثانول من البكتريا السالبة الجرام. أوضح التحليل الكيميائي الكيفي لمستخلص الزنجبيل بالميثانول أن المستخلص يحتوي على Alkaloids و Flavonoids و التي لها معدلات تأثير واسعة كمضادات للبكتيريا. كما أظهر منظار التحليل الطيفي وجود ١٦ مادة اهمها gingerol و cis-6-shagol و zingerone و 2,4-dimethyl- Benzo[h] quinolone. أظهر الاختبار التأزري تحسن في النشاط ضد بكتيري لكل من amoxicillin و gentamycin بعد اضافة مستخلص الزنجبيل بالميثانول و كان هذا التأثير أكثر وضوحا مع gentamycin. تم دراسة تأثير مستخلص الزنجبيل بالميثانول على المستوى الجزيئي بتحديد التغير في نموذج بروتين البكتريا و التغير في DNA البكتريا حيث أظهر اختبار SDS-PAGE و بشكل واضح التعدد الشكلي لمظهر بروتينات البكتريا بعد تعرضها لمستخلص الزنجبيل بالميثانول و كذلك أظهر اختبار RAPD-PCR وجود تغير في الحامض النووي للبكتيريا. ولذلك يمكن استخدام مستخلص الزنجبيل بالميثانول كبديل للمضادات الحيوية ضد البكتريا أيضا كمدعم للمضادات الحيوية التي تلاقى مقاومة من البكتريا.