Original Article

Investigating the Effects of Ginger on Biofilm Production From Bacteria Isolated From Respiratory Tract

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ABSTRACT

Background: Respiratory tract infections (RTIs) are a significant cause of morbidity and mortality worldwide. Generator workers are particularly at risk of RTIs due to exposure to air pollutants and chemical hazards. Treatment of these infections is becoming more difficult due to the growing problem of drug resistance. Both antibacterial and anti-inflammatory purposes can be achieved using ginger (*Zingiber officinale*).

Objectives: This study assesses how ginger extracts prevent and inhibit the spread of pathogens isolated from sputum samples from generator workers with RTIs.

Methods: The preparation and characterization of subcritical alcoholic and water extracts of ginger were done using gas chromatography mass spectrometry analysis. An agar-well diffusion method compared six bacterial isolates from generator workers and six bacterial isolates from non-generator workers for their antibacterial activity against the extracts. The extracts were assessed against the same isolates by using the microtiter plate assay.

Results: The ginger extracts exhibited significant antibacterial activity against all tested isolates. The extracts had a minimum inhibitory concentration (MIC) that could be measured from 0.31 to 20 mg/mL. Against all of the tested isolates, the ginger extracts showed significant antibiofilm activity.

Conclusion: Ginger extracts can be used as a natural alternative or adjunct to antibiotics to treat and prevent RTIs in generator workers.

Keywords: Ginger, Respiratory tract infection (RTIs), Biofilm, Alcoholic extract of ginger, Water extract of ginger

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Introduction

Respiratory tract infections (RTIs) are caused by the invasion of the respiratory tract by infectious microorganisms, such as bacteria or viruses (Eccles et al., 2007). Outdoor air pollution is one of the main causes of death and disease worldwide, and an estimated 4.2 million people get death risk around the world (Hulke et al., 2012).

The chemical hazards of petroleum products and their wastes are causing numerous health issues, particularly for petrol gas station workers who are exposed every day (Sherwood, 2013).

Drug resistance to multiple antibiotics in pathogens is an increasing cause of morbidity and mortality (Naeem et al., 2019). The formation of virulence factors is a significant health concern due to the difficulty in preventing and controlling them (Samiappan et al., 2020). Biofilm is one of these factors that leads to several problems depending on the components of species and host defense. Bacteria can withstand extreme environmental conditions and drugs due to these reasons. An alternative approach is expected to be developed to treat these infections (Kim et al., 2006).

The utilization of natural resources has become a new trend due to its less harmful effects. For instance, the extract of ginger (*Zingiber officinale*) is full of many components that have high antioxidant activity (Zai et al., 2021). Ginger's phytochemicals have been found to have antioxidant, anti-inflammatory, and potential cancer-preventing properties (Oosthuizen et al., 2019).

Accordingly, this study analyzes the subcritical alcoholic and water extracts of ginger (*Z. officinale*) and assesses their antibacterial growth and antibiofilm properties toward pathogens that are recovered from sputum samples from generator workers with RTIs.

Materials and Methods

Preparation of ginger extract

Z. officinale used in the present study was purchased from the local market (Al-Shourga) in Baghdad, Iraq. The fresh ginger rhizomes were washed, peeled, sliced, and air-dried for 21 days. After drying, ginger was ground to a fine powder using an electric blender, then, 10 g of each ginger powder was soaked (to prepare the aqueous and methanol extracts) in 100 mL distilled water and 100 mL of methanol separately. The two flasks were incubated at room temperature for 72 h. The crude extracts were centrifuged at 3000 rounds per min for 10 min at 28 ˚C, and the supernatant was filtered with filter paper, the extracts were concentrated using a rotary evaporator. This method was done according to Pius et al., 2015.

Gas chromatography mass spectrometry analysis (GC-MS)

A GC-MS analysis was done in the Ministry of Science and Technology by using the mass spectrophotometer Shimadzu GC-2010 Plus coupled with Shimadzu GCMS-Q2010 Ultra (EAI company/ USA). Features of the capillary column were (inert cap 1 MS, 0.25 mm, 30 m, 0.25 µm, Gl Sciences, Japan). The carrier gas was helium, the constant flow rate was 1 mL/min, the auto-injector was AOC-20i, Shimadzu, the injection volume was 5 µL, and the column oven temperature was 100 ˚C.

The identification of chemical components was a direct comparison of the retention times and mass spectral data with computer matching with the NIST mass spectral search program for the NIST/EPA/NIH mass spectral library version 2.0 f / 2008 to identify known compounds in the crude extract by comparative analysis of the obtained peaks.

Samples collection

A total of 184 sputum samples were collected from generator workers who were suspected of lower respiratory tract infection in different places in Baghdad, Iraq, and 50 sputum samples from non-workers in generators (control) were collected from three hospitals in Baghdad City, Iraq. Both groups were collected from men only and from different ages. All samples were taken to laboratory to culture and identification.

Identification of bacterial isolates

Sputum specimens were stained by gram stain and examined under a light microscope, presence of less than ten squamous epithelial cells and more than 25 leucocytes (or pus cells) for each as shape, arrangement and biochemical reactions (LPF) ensured the reliability of the specimen, which means was not saliva contaminated (Santella et al., 2020).

Sputum specimen was cultured on MacConkey agar, blood agar, and chocolate agar, and re-cultured until pure colonies; then isolates were examined microscopically and Identification tests were done including cultural mor-

phology and physiological characteristics of each bacterial isolate as mentioned by Forbes et al., 2007. Finally, the VITEK® 2 Compact system was dedicated to confirming the identification of significant bacteria clinically.

Inhibitory activity test

Both ginger extracts (methanolic and aqueous) at different concentrations (400, 200, 100, and 50 mg/mL) were used for antimicrobial activity by deep-well agar diffusion method using Muller Hinton agar as mentioned by Olayemi and Opaleye, 1999.

Screening of biofilm forming bacteria

Detection of isolates' ability to form biofilm was done by microtiter plate assay performed by Babapour et al., 2016.

Detection of the bioactive constitutes of ginger extracts

Glycosides

The presence of glycosides in ginger extracts was detected by mixing 2 mL of ginger extract with 1 mL of each glacial acetic acid, $FeCl_3$, and H_2SO_4. The presence of a green-blue color means the presence of glycosides (Trease & Evans, 1989).

Alkaloids

The presence of alkaloids was detected by adding extract (2 mL) to hydrochloric acid (1 mL) and then adding a few drops of picric acid solution. A grainy gray color means the presence of alkaloids (Harbone, 1973).

Tannins

The presence of tannins in ginger extracts was detected by adding drops of $FeCl_3$ solution (1%) to 0.5 mL of each extract in a test tube, the presence of bluish green color meant the presence of tannins (Burns, 1971).

Phenols

The presence of phenols in ginger extracts was detected by using a ferric chloride solution (prepared by dissolving ferric chloride salt in distilled water). This reagent gives a blue or green color when added to the extract if it contains phenolic compounds (Harbone, 1973).

Saponin

The presence of saponin in ginger extracts was detected by adding 1 to 3 mL of mercuric chloride solution ($HgCl_2$ 1%) to 5 mL of extract. The appearance of a white precipitate indicates a positive result (Shihata, 1951).

Flavonoids

The presence of flavonoids in ginger extracts was detected by mixing each ginger extract (2 mL) with diluted hydrochloric acid and diluted NaOH. The appearance of yellow solution color indicates the presence of the flavonoid (Jaffer et al., 1983).

Terpenes and steroids

The presence of terpenes and steroids in ginger extracts was detected by mixing each ginger extract (1 mL) with a small amount of chloroform, filtered, then adding a drop of acetic anhydrate and a drop of concentrated sulfuric acid to the 2 mL of filtrate as mentioned before. The presence of brown color indicated the presence of terpenes, then after leaving the mixture for the period, the blue-green ring indicated the presence of steroids (Al-Abid, 1985).

Determination of minimum inhibitory concentration (MIC) of ginger extract

Two-fold serial dilutions were done in a range of concentration between 0.019-20 mg/mL from stock (0.5 g/10 mL) of ginger extract from both (methanolic and aqueous) extracts in Mueller–Hinton broth as diluent.

All wells were inoculated with 20 µL of bacterial suspension (compared with 0.5 McFarland's standard except the control wells). Microtiter plates were incubated at 37 °C for 18 to 24 h. After incubation, 20 µL of resazurin dye was added to all of the wells and incubated for another 2 h for detection of any color changes. The sub-MIC was defined visually in broth microdilutions as the lowest concentration at which color changed from blue to pink as mentioned in the resazurin broth assay (Ohikhena et al., 2017).

The antimicrobial assay of ginger extracts using agar-well diffusion method

The antimicrobial assay of both ginger extracts (methanolic and aqueous) at different concentrations (400, 200, 100, 50 mg/mL) was done by deep-well agar diffusion method as mentioned by Olayemi and Opaleye, 1999.

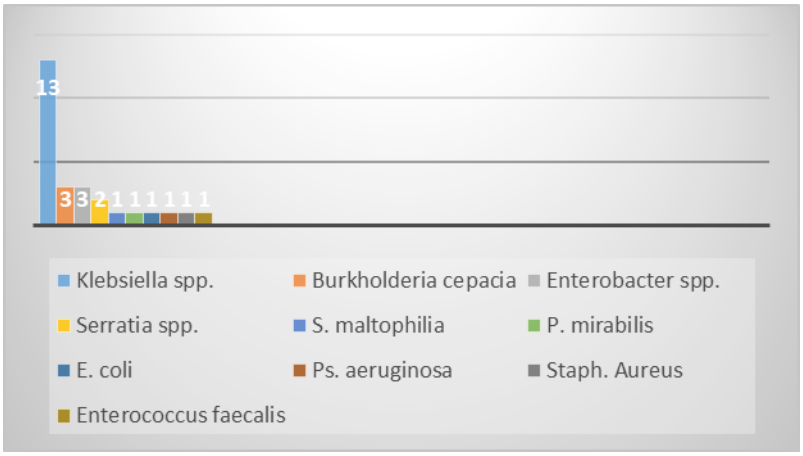

Figure 1. Bacterial isolates of generator workers according to VITEK-2 system results

Antibiofilm test by ginger extracts

The same protocol was used for the biofilm formation assay, which was previously mentioned for screening of biofilm forming bacteria. After the preparation of sterilized brain heart infusion broth with 2% sucrose, 180 µL of brain heart infusion broth (BHIB) was mixed with each ginger extract (methanolic and Aqueous) sub-MIC concentrations, then added to each well, 20 µL of bacterial suspension (compared to 0.5 MacFarland) was introduced, whereas the control contained just 180 µL of BHIB and 20 µL of bacterial suspension, then complete the steps mentioned by (Babapour et al., 2016).

The methanolic and aqueous ginger extract were in a range of concentrations for each extract between 20, 10, 5, 2.5, 1.25, 0.62, 0.31, 0.15, 0.07, 0.03, and 0.019 mg/mL against six bacterial isolates from generator workers group (*Burkholderia cepacia* No. 14, *Klebsiella pneumoniae* No. 25, *Proteus mirabilis* No. 28, *Serratia marcescens* No. 92, *Enterobacter aerogenes* No. 97, and Enterobacter faecalis No. 162), in addition to another six bacterial isolates from non-generator workers group, namely Acinetobacter baumannii No. 15, *Pseudomonas aeruginosa* No. 17, *K. pneumoniae* No. 26, *A. baumannii* No. 31, *A. baumannii* No. 36, and *A. baumannii* No. 38.

Results

The work duration for the generator workers was between 4 to 18 years with a Mean±SD of 9.2±1.1 years and the ages of generator workers were between 26 to 59 years with a Mean±SD of 39.7±1.86 years. Non-generator workers were asked to confirm that no working history in this field and ages were between 22 to 57 years with a Mean±SD of 36.5±9.8 years. From 184 sputum samples of generator workers, there were 27 samples resulted in significant bacterial growth (14.67%), composed of 25 isolates gram-negative bacterial isolates (92.59%) and two isolates were gram-positive (7.40%) as shown in Figure 1.

Out of 50 sputum samples of non-workers in generators (control), 27 samples resulted in significant bacterial growth (54%); however, all bacteria were gram-negative (100%) as shown in Figure 2. Diagnosis of the bacterial isolates of both studied groups of samples was confirmed by the VITEK-2 system.

The bacterial species that were isolated from sputum samples (184 generator workers + 50 sputum samples from non-generator workers) were *Klebsiella* spp., *B. cepacia*, *Enterobacter* spp., *Serratia* spp., *Stenotrophomonas maltophilia*, *P. mirabilis*, *Escherichia coli*, *P. aeruginosa*, *Staphylococcus aureus*, *A. baumannii*, and *Enterobacter cloacae* as shown in Figure 1 for generators workers and in Figure 2 for non-generator workers.

Concerning biofilm formation the criteria were listed in Table 1, the present study maintained that there were significant differences between isolates that isolated from workers and non-workers in electrical generators at $P<0.05$ for biofilm formation, 2(7.4%) and 0(0%) strains were classified as non-biofilm formers, 17(62.9%) and 15(55.6%) as weak biofilm former, 2(7.4%) and 6(22.2%) as moderate biofilm former, and both of them 6(22.2%) as strong biofilm former for generator workers and non-workers respectively.

Also, in our study, the chemical composition of each extract (methanolic and aqueous) of ginger was analyzed by GC-MS, and the results were listed in Table 2 and Table 3.

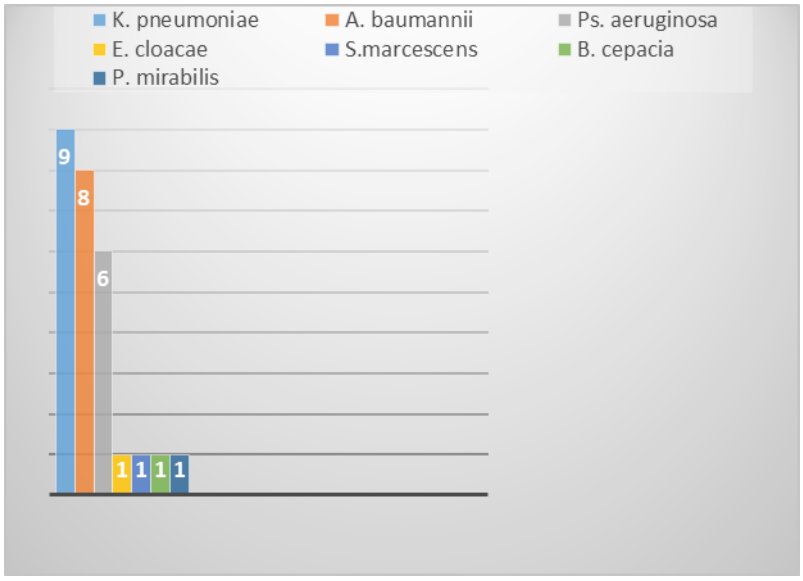

Figure 2. Bacterial isolates from non-workers in generators according to VITEK-2 system results

The results of GC-MS analysis of the methanolic extract of ginger recorded in Table 2 showed that as 26 compounds were obtained and identified, the most materials that had significant activities in this study were oleic acid (25.28 %), followed by diethyl phthalate by 10.46%, pentadecanoic acid (8.68%), 13-octadecyl (5.48%), gingerol (5.20%), hexadecenoic acid methyl ester (2.06%), cis-β Farnesene (1.93%), capsaicin (1.79 %), and gitoxigenin (1.64%).

In addition, based on the results of GC-MS analysis of Aqueous extract of ginger in Table 3, as 30 compounds were obtained and identified, the most important materials that had significant activities in this study were 9-octadecenoic acid (Z)- methyl ester by 9.66%, 1,3-cyclohexadiene, 5(1,5-dimethyl-4-hexenyl)-2-methyl-,[S-(R*, S*), gingerol by 8.17%, benzene acetic acid, 4-hydroxy-3-methoxy-, methyl ester by 7.24%, oleic acid by 6.79%, and gamma-Muurolene by 5.88%), β-sitosterol (4.69%), hexadecenoic acid methyl ester by 3.60%, lanosterol by 2.598%, folic acid by 1.24%, diethyl phthalate by 1.17%, and 2-dodecenal by 1.09%.

For the active compounds in ginger extract, there were chemical components in methanolic and aqueous extract of ginger which are secondary metabolism products, as shown in Table 3

The ginger extract effect on bacterial isolates was studied by broth microdilution method in a 96-well microtiter plate for determination of the MIC of ginger extract by different concentrations against six strong biofilm former bacterial isolates from each of generator workers group and non-generator workers group. The results revealed that the methanolic and aqueous ginger extracts were effective against both generator workers' isolates and non-generator workers' isolates.

Table 1. Categories of biofilm formation for bacterial isolates

Biofilm Production	No. (%)	
	Generator Workers Bacterial Isolates	Non-generator Workers Bacterial Isolates
Non producer	2(7.4)	0(0)
Weak	17(62.9)	15(55.6)
Moderate	2(7.4)	6(22.2)
Strong	6(22.2)	6(22.22)
P	0.001*	0.043*

*Significant difference at P<0.05.

Table 2. Results of chemical constitutes for ginger methanolic and aqueous extracts as active compounds

Active Compounds	Methanolic Extract	Aqueous Extract
Phenols	+	+
Alkaloids	-	-
Glycosides	-	+
Tannins	+	-
Saponins	-	-
Flavonoids	+	-
Steroids	-	+
Terpenes	+	+

The results recorded that the sub-MIC concentrations which affected the strong biofilm former of the generator workers group of methanolic extract were 1.25, 0.62, 0.31, 0.62, 1.25, and 2.5 mg/mL, and for aqueous extract were 5, 2.5, 1.25, 2.5, 5, and 10 mg/mL.

For non-generator workers sub-MIC concentrations for a methanolic extract of ginger were 0.31, 2.5, 2.5, 0.62, 5, and 1.25 mg/mL, and for aqueous extract of ginger was 2.5, 10, 10, 2.5, 10, and 5 mg/mL as shown in Table 4.

Additionally, the ginger extracts' antibacterial activity was done by agar-well diffusion method for methanolic and aqueous extracts of ginger according to the concentration of MIC of each studied group, three replicates were done for each bacterial isolate.

The MIC concentration used for electrical generator workers bacterial isolates of methanolic extract were 2.5, 1.25, 0.62, 1.25, 2.5, and 5 mg/mL, while for aqueous extract were 10, 5, 2.5, 5, 10, and 20 mg/mL; concerning non-generator workers bacterial isolates the MIC concentrations used of methanolic extract were 0.62, 5, 5, 1.25, 10 and 2.5 mg/mL, while for aqueous extract were 2.5, 10, 10, 2.5, 10, and 5 mg/mL as described in Table 5 to inhibit the growth of bacteria.

The results demonstrated that the tested bacterial isolates were more sensitive for both used aqueous and methanolic extracts of ginger; furthermore, higher sensitivity of bacterial isolates was for methanolic extract compared with the aqueous extract, as triple of each bacterial isolate than that for the aqueous extract, this proved by recording higher diameters of inhibition zones, especially by recording significant differences at $P<0.001$ and $P<0.05$ between methanolic and aqueous extract of ginger for both studied groups as shown in Table 5 and Table 6.

In both studied groups, strong biofilm former bacterial isolates were subjected to sub-MIC concentrations of ginger extract (methanolic and aqueous extracts). The results showed a reduction of bacterial biofilm formation as shown in Table 6. The optical density (OD) of biofilm before treatment was significantly higher at the level ($P<0.001$) in all bacterial isolates, in generator workers' bacterial isolates before treatment Mean±SD were 0.34±0.01, 0.37±0.02, 0.35±0.01, 0.34±0.01, 0.38±0.01, and 0.36±0.02 as compared with methanolic extract were 0.11±0.02, 0.51±0.02, 0.15±0.02, 0.11±0.02, 0.26±0.01, and 0.26±0.01, while in aqueous extract were 0.72±0.12, 0.63±0.36, 0.23±0.12, 0.06±0.005, 0.14±0.05, and 0.12±0.11.

For non-generator workers bacterial isolates the OD before treatment Mean±SD were 0.45±0.01, 0.37±0.02, 0.41±0.01, 0.43±0.01, 0.47±0.02, and 0.47±0.02 as compared with methanolic extract were 0.23±0.02, 0.16±0.01, 0.23±0.02, 0.21±0.01, 0.23±0.01, and 0.27±0.01 in aqueous extract were 0.10±0.03, 0.08±0.02, 0.17±0.01, 0.18±0.01, 0.08±0.01, and 0.16±0.01 (Table 7); therefore, the methanolic extract inhibit the biofilm formation more than the aqueous extract. This was confirmed by the significant differences of all bacterial isolates for both studied groups at the level ($P<0.001$).

Discussion

RTIs are among the most common diseases, as reported by various studies (Jin et al., 2021). There were 13 isolates (48.14%) from generator workers and 3 isolates (33.33%) from non-workers in generators that were

Table 3. Determination of MIC of ginger extracts against bacterial isolates from workers and non-workers in generators

Generator Workers Bacterial Isolates	Methanolic Extract		Aqueous Extract	
	MIC (mg/mL)	Sub-MIC (mg/mL)	MIC (mg/mL)	Sub-MIC (mg/mL)
B. cepacian, No. 14	2.5	1.25	10	5
K. pneumoniae, No. 25	1.25	0.62	5	2.5
P. mirabilis, No. 28	0.62	0.31	2.5	1.25
Serratia marcescens, No. 92	1.25	0.62	5	2.5
E. aerogenes, No. 97	2.5	1.25	10	5
Enterococcus faecalis, No. 162	5	2.5	20	10
Non-workers in Generator Bacterial Isolates	**Methanolic Extract**		**Aqueous Extract**	
	MIC (mg/mL)	**Sub-MIC (mg/mL)**	**MIC (mg/mL)**	**Sub-MIC (mg/mL)**
Acinetobacter baumannii, No. 15	0.62	0.31	5	2.5
Pseudomonas aeruginosa, No. 17	5	2.5	20	10
K. pneumoniae, No. 26	5	2.5	20	10
Acinetobacter baumannii, No. 31	1.25	0.62	5	2.5
Acinetobacter baumannii, No. 36	10	5	20	10
Acinetobacter baumannii, No. 38	2.5	1.25	10	5

MIC: Minimum inhibitory concentration.

preliminarily identified as K.pneumoniae out of the total samples as shown in FigureS 1 and 2. Other studies have shown that Klebsiella can cause a variety of infections, and since it belongs to Enterobacteriaceae, it is a threat to nosocomial infections (Zhu et al., 2021). When we classified the strains into biofilm-forming and non-biofilm-forming, it was observed that non-generator workers had more biofilm-forming strains than the other group of isolates (Balle'n et al., 2021).

Concerning compound named as octadecenoic acid(Z)-methyl ester, it owned antibacterial and antibiofilm activity (Ghareeb et al., 2022), while 1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*) had

Table 4. Antibacterial activity of methanolic and aqueous extract on generator worker's bacterial isolates measured by mm

Generator Workers Bacterial Isolates	Mean±SD		P
	Methanolic Extract	Aqueous Extract	
B. cepacia No. 14	20±0.56	15.3±0.58	<0.001**
K. pneumoniae No. 25	17.3±0.57	14.3±0.56	0.003**
P. mirabilis No. 28	17.1±0.61	11.3±0.52	<0.001**
S. marcescens No. 92	13.6±0.57	12.7±0.57	0.045*
E. aerogenes No. 97	17.6±0.57	14.6±0.56	0.003**
Enterococcus faecalis No. 162	16.6±0.51	14.6±0.53	0.013*

SD: Standard deviation.

Table 5. Antibacterial activity of methanolic and aqueous extracts on non-generator workers bacterial isolates measured by mm

Non-Workers in Generators Bacterial Isolates	Mean±SD		P
	Methanolic Extract	Aqueous Extract	
A. baumannii No. 15	12.3±1.15	9.6±0.57	0.023*
P. aeruginosa No. 17	21.3±0.57	17.3±1.15	0.006**
K. pneumoniae No. 26	25.6±0.58	17.6±0.57	0.001**
A. baumannii No. 31	15.3±0.55	12.6±0.56	0.005**
A. baumannii No. 36	23.3±0.57	13.6±0.56	0.001**
A. baumannii No. 38	17.7±0.57	16.3±0.56	0.047*

SD: Standard deviation.

*Significant difference at P<0.05, **Significant difference at P<0.001.

antibacterial activity (Ohaegbu et al., 2022), gingerol effect as antimicrobial and antibiofilm agent (Riaz et al., 2011), benzene acetic acid, 4-hydroxy-3-methoxy-, methyl ester (7.24%) had antibacterial activity (Shareef et al., 2016), besides oleic acid (6.79%) which had also antibacterial activity (Abitogun & Badejo, 2010), gamma-Muurolene (5.88%) effect as antioxidant and antimicrobial agent (Mutlu-Ingok et al., 2021), beta-sitosterol (4.69%) had both antimicrobial and antibiofilm activity (Dogan et al., 2017), and lower compound were hexadecenoic acid methyl ester (3.60%) which effect as antibiofilm, antibacterial, antimicrobial and antioxidant agent (Hamad et al., 2016), lanosterol (2.598%) had antibacterial activity (Mathew et al., 2022), folic Acid (1.24%) had also antibacterial activity (Shihata, 1951), diethyl phthalate (1.17%) had antibacterial, antimicrobial and antibiofilm activity (Rashiya et al., 2021) and 2-dodecenal (1.09%) had antimicrobial activity (Daniel-Jambun et al., 2017).

The many compounds present in ginger are referenced by many sources (Chakotiya et al., 2018), The positive effects of medicinal plant extracts are probably related to the antimicrobial effects of the active components in their composition (Gholipour-Shoshod et al., 2023).

The findings indicated that the methanolic and aqueous ginger extracts had a positive impact on both generator worker isolates and non-generator worker isolates

Table 6. Antibiofilm activity of methanolic and aqueous extracts of ginger on generator worker's bacterial isolates

Generators Workers Bacterial Isolate	Mean±SD			P
	Biofilm Formation Before Treatment	Biofilm Formation After Treatment		
		Methanolic Extract	Aqueous Extract	
B. cepacia No. 14	0.34±0.01	0.11±0.02	0.72±0.12	0.001**
K. pneumoniae No. 25	0.37±0.02	0.51±0.02	0.63±0.36	0.001**
P. mirabilis No. 28	0.35±0.01	0.15±0.02	0.23±0.12	0.002**
S. marcescens No. 92	0.34±0.01	0.11±0.02	0.06±0.005	0.004**
E. aerogenes No. 97	0.38±0.01	0.26±0.01	0.14±0.05	0.003**
E. faecalis No. 162	0.36±0.02	0.26±0.01	0.12±0.11	0.005**

**Significant at P<0.001.

Notes: Greenhouse-Geisser was used to compare repeated measures of the variable of probability.

Table 7. Antibiofilm activity of methanolic and aqueous extracts of ginger on non-generator worker's bacterial isolates

Non-Generators Workers Bacterial Isolates	Mean±SD			P
	Biofilm Formation Before Treatment	Biofilm Formation After Treatment		
		After Treatment	Aqueous Extract	
A. baumannii No. 15	0.45±0.01	0.23±0.02	0.10±0.03	0.002**
P. aeruginosa No. 17	0.37±0.02	0.16±0.01	0.08±0.02	0.002**
K. pneumoniae No. 26	0.41±0.01	0.23±0.02	0.17±0.01	0.005**
A. baumannii No. 31	0.43±0.01	0.21±0.01	0.18±0.01	0.001**
A. baumannii No. 36	0.47±0.02	0.23±0.01	0.08±0.01	0.001**
A. baumannii No. 38	0.47±0.02	0.27±0.01	0.16±0.01	0.002**

**Significant at P<0.001.

Notes: Greenhouse-Geisser was used to compare repeated measures of the variable of probability.

and the bacteria's growth can be inhibited by a higher concentration of aqueous extract than that of methanolic extract.

As described in Table 4, previous studies have shown that methanolic extract has lower MIC concentrations than aqueous extract and this was compatible with previous studies of (Yassen & Ibrahim, 2016) who pointed out that the concentration of methanolic ginger extract was not as strong as that of aqueous extract, which is necessary for inhibiting bacterial growth, Both methanolic and aqueous extracts of ginger, as previously mentioned and explained, could account for this activity.

The component found in methanolic and aqueous extracts may be the cause of ginger extract's inhibitory effect, as indicated by the recent study e.g. methanolic extract had phenols, tannins, saponins, terpenes, and flavonoids, while aqueous extract had phenols, glycosides, terpenes, and steroids as recorded by GC-MS analysis results.

Methanolic and aqueous ginger extracts contained phenolic compounds, which were considered anti-bacterial agents and had an inhibition effect on bacteria's growth. They had multiple roles in numerous metabolic enzymes, which led to the disruption of critical processes and the death of bacteria (Kumar et al., 2014). Furthermore, tannins exert antibacterial effects by blocking the transport of proteins and enzymes in the cell membrane, breaking down the cell membrane, and inhibiting proteolytic enzymes (Udu-ibiam et al., 2014)

In addition, saponins can lead to damage to the bacterial cell membrane by exuding substances like nucleic acids, proteins, and nucleotides, which may result in bacterial lysis. In addition, saponins can lead to damage to the bacterial cell membrane by exuding substances like nucleic acids, proteins, and nucleotides, which may result in bacterial lysis (Effiom et al., 2021).

Flavonoid binding to the bacterial lipid bilayer results in membrane damage and inhibition of extracellular and intracellular enzyme synthesis (Reygaert, 2014). Terpenes have not been clearly defined in their actions, but they influence the bacterial cell membrane and virulence factors, such as efflux pump modulation (Barbieri et al., 2016). The ability to break the bacterial cell and interfere with DNA is present in glycosides (Dias et al., 2021), in addition, lipids of bacterial membranes have sensitivity against steroidal compounds which cause leakage disruption in the cell membrane (Epand et al., 2007), all these active components had increased the inhibitory effects of ginger extracts against respiratory bacteria (Kodikara et al.,2022).

Biofilm is a consortium of various microorganisms that is a major source for the formation of infection, several researchers recorded an increase in the prevalence of infection following multidrug-resistant bacterial isolates, this inhibitory activity of biofilm formation resulted from the bioactive compounds extracted using methanol as solvent (Samiappan et al., 2020). The bacterial ability to form biofilm is responsible for the establishment of a persistent infection (Foroutan et al., 2021), Besides that, the nosocomial bacterial isolates were more resistant to

antimicrobial agents, which in turn increased the difficulties of treatment (Meamar et al., 2021).

The ability of the ginger extract to inhibit the growth of biofilm former bacteria provided us with a new insight into the antimicrobial properties of this herb (Oosthuizen et al., 2019). These bioactive compounds include 6-gingerol, 6- shogaol, zingerone, etc. were identified in both extracts, in some times the subcritical water extract of ginger was more efficient in removing biofilms of some biofilm former bacteria, this observation can be resulted from the presence of antimicrobial compounds such as 6-shagaol and zingerone in gingers subcritical water extract, which destroyed the biofilms, on other hand, lower bioactive compounds were presented in the aqueous ethanolic extract of ginger like peracetic acid, implying the highest antibiofilm activity (Oosthuizen et al., 2019), this feature is critical in the development of drugs used to treat biofilm- related infectious disease because it reduces the antibiotic-resistant bacteria (Kim & Park, 2013), other studies explain the activity of ginger extract on biofilm formation by that ginger extract inhibited biofilm formation by lowering the level of cellular C-di-GMP, furthermore, the addition of ginger extract reduced biofilm formation in a PA mutant over produces cellular C-di-GMP which confirmed the relevance of ginger extract to lowering cellular level of C-di-GMP (Pius et al., 2015).

Numerous studies have demonstrated the role of C-di-GMP as a global second messenger across diverse bacteria including gram-positive and gram-negative bacteria, C-di-GMP reportedly modulates bacterial physiology and behavior, including motility, virulence, and biofilm formation through transcription, translation, all these mechanisms may be due to the presence of constituents like tannin, terpenoid, saponin and flavonoids (Pramiastuti et al., 2018). Therefore, ginger has a high total phenolic content that leads to very potent antioxidant activity (Kusriani et al., 2017). Moreover, phenolic compounds and flavonoids are formed by many plants and fruit species and consumed in traditional medicine or diets, they have antimicrobial activity (Karamati Jabehda et al., 2021).

Ethical Considerations

Compliance with ethical guidelines

All ethical principles are considered in this article. The participants were informed of the purpose of the research and its implementation stages. They were also assured about the confidentiality of their information and were free to leave the study whenever they wished, and if desired, the research results would be available to them. A written consent has been obtained from the subjects. Principles of the Helsinki Convention was also observed.

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Authors' contributions

Methodology, and formal analysis: Huda Zuheir Majeed; Data curation, review and editing: Yusra Mohmed Baqer Muhsin, Investigation, Resources, review and editing: Rasha Mohmmed Sajet.

Conflict of interest

The authors declared no conflict of interest.

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