Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 3, 475-480

Antibacterial activity of plant phytochemicals on antibiotic-resistant bacteria isolated from meat

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ARTICLE INFO

Recieved: 17 November 2023

Accepted: 08 January 2024

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Keywords:

Multidrug resistance (MDR)
Minimum inhibitory concentration (MIC)
Thin-layer chromatography (TLC)
Gas chromatography-mass spectrometry (GC-MS)

ABSTRACT

Multidrug resistance poses a danger to public health and steadily raises mortality, despite the discovery and development of a wide range of antimicrobial drugs. Developing practical solutions to address these problems has been the focus of many recent studies. In this work, we elucidate the way certain antibiotics, when coupled with other antimicrobial agents like plant extracts, can produce synergistic effects provided that each therapy targets a separate target or signaling pathway and functions via a different mechanism. Gas chromatography-mass spectrometry (GC-MS) and thin-layer chromatography (TLC) were used to identify bioactive metabolite extracts and purify plant extracts with active antibacterial qualities. The minimum inhibitory concentration (MIC) was determined using the broth microdilution method. Different 100 samples of meat (fresh, frozen Brazilian, Sudanese, and minced meat) and meat products such as pastrami, burgers, kofta, luncheon, and sausage were randomly collected from six (6) different locations in Al-Sharkia Governorate, Egypt. The associated pathogenic bacteria recovered from the isolated raw meat samples identified by microbiological, biochemical, and molecular methods is *Bacillus*. Clove extract was found to be the most effective and exhibited bacteriostatic and bactericidal activities against highly resistant strains of pathogenic bacteria. The plant extracts used in the study have great efficacy in reducing bacterial contamination and can be used as a safe alternative to antibiotics to avoid and control foodborne diseases.

Introduction

In addition to hospitals and other healthcare facilities, MDR species can be found in people, animals, plants, food, water, soil, and air. Antibiotic-resistant illnesses claim the lives of almost a million people worldwide. If treatments are not developed, antibiotic-resistant infections may account for 10 million fatalities by 2050 and the issue will still not be under control (Hashempour *et al*., 2019). Meat is one of the main sources of the key elements needed for healthy growth and maintenance, including proteins, vitamins, and minerals (Orpin *et al*., 2018). Multi drug resistant (MDR) bacteria have been found on meat products in a number of international investigations (Ateba., *et al* 2008; Landers *et al*., 2012; Asiimwe *et al*., 2017; Djeffal *et al*., 2018; Lee *et al*., 2020). One of the main sources of food borne illnesses and a significant source of zoonotic infections are tainted meats (Ali *et al*., 2010).

Bacterial resistance is the ability of bacteria to withstand the inhibitory or destructive effects of an antibiotic to which it was not resistant is referred to as resistance (Dehbanipour *et al*., 2016; WHO, 2019; Motse *et al*., 2019). This adaptive process is primarily brought on by the bacterial enzymatic degradation of antibiotics, the mutation of the antibiotic target, the change in membrane permeability, and alternative metabolic pathways (Arsene *et al*., 2022).

Furthermore, although smaller dosages are ineffective, the host organism usually cannot withstand the overuse of antibiotics. Antibiotics can become less or even useless when bacteria develop a tolerance to them due to prolonged use, increased usage, and abuse. Additionally, commonly used antibiotics promote the growth of antibiotic resistance in live microorganisms (Beyth *et al*., 2015), and one reason for this is altering the targets of antibiotics (Baym *et al*., 2016).

Using various antimicrobial agents such as plant extracts in conjunction to create synergistic effects is an alternate approach that is presently used or being tested (Kaur, 2016). Combination therapy is a preferred and elective course of treatment since it reduces the risk of cross-resistance development and offers potential adjuvant targets of other signalling pathways. (Bozic *et al*., 2013).

Throughout the history, plants and their extracts have been employed for therapeutic purposes. Secondary metabolites, such as alkaloids, terpenoids, flavonoids, and tannins, have the ability to be produced by plants and have a wide range of organic molecules with a wide structural diversity (Joachim *et al*., 2018). The safety of herbal plants is considered as one of their most significant benefits, along with their affordability, potency, and accessibility (Siddiqui, 1993).

Consequently, the purpose of this study was to investigate the antibacterial properties of plant extracts to determine how well they worked in conjunction with conventional antibiotics to treat a variety of multi drugs resistant bacteria.

Materials and methods

Collection of Samples

Different samples of meat and meat products were randomly obtained from six different locations in Al-Sharkia governorate. A total of 100 samples from meat (fresh, frozen Brazilian, Sudanese, and minced meat) and meat products such as (pastrami, burger, kofta, luncheon and sausage) were collected from different locations in Al-Sharkia governorate collected from supermarkets and butcher shops during the period 1/3/2022 until 1/6/2022 in different level of sanitation.100 g of meat sam-

ISSN: 2090-6277/2090-6269/

ples were procured and put into sterile, dry, and clean polythene bags before being delivered to the lab for microbiological examination.

Culture Conditions for Isolation of Bacteria

Using a sterile knife, the samples were aseptically divided into small, thin pieces. 250 mL of distilled water were used as the stock after the analytical portions were homogenized in separate sterile plastic bags. For the serial dilution experiment, 1 mL of stock homogenate and 9 mL of sterile distilled water were used to achieve serial dilutions up to fivefold (10-5) for each prepared sample. In order to obtain a distinct colony, this was done. We used the spread plate approach to pour pre-serially diluted samples (0.1 mL) onto ready-made solidified nutrient agar plates. This was given five (5) minutes to set thoroughly before inoculation plates were incubated at 37°C for 24 hours. Following a 24-hour observation period, bacteria colonies were sub-cultured onto freshly made nutrient agar in sterile petri dishes to produce distinct colonies, which were then identified (Oluwatobi *et al*., 2021).

Antibiotic sensitivity test

The isolates were subjected to a Kirby Bauer's disc diffusion method antibiotic sensitivity test on Nutrient Agar (NA) using antibiotic discs that were readily accessible commercially. Using a sterile cotton swab dipped in bacterial suspension, a grass culture was performed on the surface of a NA plate after standardization. Antibiotic discs that were easily purchased were put on the surfaces of the contaminated plates. At 37°C, the plates were incubated for 16–18 hours. After incubation, each antimicrobial drug's zone of inhibition was evaluated, and the results were compared to the NCCLS chart (Oluwatobi *et al*., 2021).

Determination of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC is the lowest antibacterial agent concentration that totally prevents bacterial growth. Using the broth microdilution method, the MIC of the various extracts was evaluated (Manga *et al*., 2021; Konstantinovitch *et al*., 2022).

Briefly, A U-bottom 96 well microplate with the first row (columns 1-10) filled with 100 L of bacterial culture. Each antibiotic dilution was added to them in 100 L. Then, as a negative control, 100 L of sterile broth devoid of culture was introduced to the well of column 11. Then a 100 L positive control of bacterial culture was introduced to the well of column 12. Finally, the plates were covered and incubated at 37°C for 24 h. After incubation, MIC was considered the lowest concentration of the tested material that inhibited the visible growth of the bacteria. MBCs were determined by subculturing the wells without visible growth (with concentrations ≥MIC) on MHA plates. Inoculated agar plates were incubated at 37°C for 24 h. MBC was considered the lowest concentration that did not yield any bacterial growth on agar. (Mbarga *et al*., 2022).

Plant material and extraction procedures

To extract alcohol Materials from 12 distinct plant species, Plant pieces were thoroughly cleansed to get rid of any potential contaminants. Each plant material was dried before being crushed into a fine powder that could pass through a 100 mm filter. 10 g of fine powder were placed in 100 mL of methanol, extracted for 48 hours while being continuously stirred, filtered using a two-layer muslin cloth, spun at a high speed for 10 minutes, and then filtered once more using Whatman filter paper (Merck Millipore, Mumbai, India). The filtrates were dried using a rotary vacuum evaporator (Hahnshin Scientific, Mumbai, India) under reduced pressure at 60°C and stored in the refrigerator at 5°C (Imran *et al*., 2021).

Thin-layer chromatography TLC

On silica gel paper chromatography, the extract was initially evaluated using Thin-Layer Chromatography (TLC) (Sigma-Aldrish, Germany). TLC was performed using a variety of solvent systems with different polarity in order to select the solvent system that might demonstrate higher resolution such as Hexane : ethyl acetate (3:9) , toluene: ethyl acetate: formic acid (7:3:0.2), methanol : Chloroform (5:4) Hexane : ethyl acetate: Glcial acetic acid (8:2:1) and ethyle acetate : Chloroform (9:5) .

After being air-dried, the resulting TLC plates were examined under a UV TLC viewer at both 254 and 366 nm. (Gaurav *et al*., 2020) computed the rate of flow (RF) value of the various places that were observed. One by one, the bands were taken off and put into separate vials. We next used the agar well diffusion method to examine each band's antibacterial activity (Abdelaziz *et al*., 2023).

GC-MS investigation

The methanolic extract of clove was subjected to GC-MS analysis using Shimadzu Japan gas chromatography QP2010PLUS with a fused GC column 2010 coated with polymethyl silicon (0.25nm x 50m) and the conditions were as follows: Programmable temperature range of 80–2000C, kept at 80 0C for 1 minute, 5 0C/min, and 200 0C for 20 minutes. Temperature of the field ionization detector (FID): 300 0C; injection temperature: 220 0C; nitrogen carrier gas flow rate: 1 ml/min; split ratio: 1:75. A GCMS-QP 2010 Plus, Shimadzu Japan gas chromatography mass spectrum was performed utilizing a carrier gas pressure of 116.9 kpa and an injector temperature of 220°C. With a 0.25 mm diameter and a 50 ml/min flow rate, the column has a length of 30 m. A mass spectrometer equipped with a 1.5 kv dictator voltage and a 0.2 sec sampling rate automatically received the elutes. Along with the mass spectrum, there was also a computerized mass spectra data bank. They used a centrifuge. Identification of a chemical ingredient By comparing the peak mass spectra to computerized Wiley MS libraries, the chemical constituent components of the extracts were identified (Saleem and Meinwald, 2000; Ogunlesi *et al*., 2010; Yang *et al*., 2012; Ayyari *et al*., 2014).

Antibacterial activity of the plant extracts

In this procedure, the extracting solvent is an organic solvent, such as a mixture of 95% methanol and water. The mixture is occasionally shaken or stirred during the extraction process, which is carried out at room temperature. (5g) of dried plant material was immersed in a solvent solution for 24 hours, depending on the sample size. Whatman filter paper number1 was used to filter this after it had been filtered using sterile muslin cloth (Madsen *et al*., 1987; Jebeen *et al*., 2008). The extracted materials were then collected. After processing, the filtrate was used immediately. By using the filter paper disc diffusion method, the extracts were evaluated on bacterial isolates (Oluma *et al*., 2004; Doughari *et al*., 2007). More details about the strongest plant extract are provided.

Determination of minimum inhibitory concentrations and minimum bactericidal concentrations of clove extract

The MIC is the lowest antimicrobial agent concentration that, after 24 hours of incubation, suppresses microbiological growth. The most potent plant extracts were used to calculate their MIC using the disc diffusion method and assess how well they controlled MDR bacterial strains. These extracts showed high antibacterial activity at 10 mg/ml.

Different concentrations of the effective plant extract (80%, 40%, 20% ,10%, 5%, 2.5%,1.25%, 0.625%, 0.3125%, 0.156%) were prepared separately by dissolving 5 mg in 8 ml of methanol, sterilized through Millipore filter and loaded their requisite amount over sterilized filter paper discs (8 mm in diameter). The pathogenic strains of bacteria were suspended in

nutrient agar and sown in sterile Petri dishes. On top of the Nutrient agar plates, the loaded filter paper discs with various quantities of the useful plant extract were positioned. The plates were incubated at 35°C for 24 hours after being chilled at 50°C for 2 hours. The concentrations of the efficient plant extracts were recorded together with the ruler measurements of the inhibitory zones.

(MBC's) of the effective plants extract Streaks were taken from the two lowest concentrations of the plant extract plates exhibiting invisible growth (from inhibition zone of MIC plates) and subcultures onto fresh sterile Nutrient (NA) plates. The plates were incubated at 35 0 C for 24 h. then examined for bacterial growth in corresponding to plant extract concentration. MBC was taken as the concentration of plant extract that did not exhibiting any bacterial growth on the freshly inoculated agar plates (Ashraf *et al*., 2018).

Combination between the antibacterial activity of antibiotics and plant extract by disc diffusion method

Synergistic tests for bacteria to assess the synergistic antibacterial efficacy, several antibiotics were alternately combined with plant extracts. This was accomplished using the disc diffusion method, however in this stage, sterile filter paper discs were coated with two antimicrobial agents: the chosen antibiotic (at the MIC value), the plant extract (at the MIC value). On the inoculated agar plates, an antibiotic and plant extract were used in conjunction. After an overnight incubation, the zones of inhibition created by the combination of antimicrobial drugs were calculated as per Lo Cantore *et al*. (2005) description: If the zones of combination treatment were greater than the zones of plant extract and the corresponding antibiotic, they were considered synergistic; if they were equal to the zones of plant extract and the corresponding antibiotic, they were considered additive; and if they were less than the zones of plant extract and the corresponding antibiotic, they were considered antagonistic (Shahabe *et al*., 2021).

Results

Isolation of pathogenic bacteria from different meat sources

Out of the 100 samples, the majority of isolated bacteria were picked out from luncheon (18.27%). However, the minimum number of isolated bacteria were obtained from Sudanese meat (4.81%). The other sources of isolated samples showed a wobbly distribution ($p<0.001$; Table 1).

Table 1. Distribution percentage and number of isolated bacteria.

Sources	Bacterial isolates	
	No.	Distribution (%)
Fresh meat (M)	7	6.73
Frozen meat (F)	14	13.46
Brazilian meat (BM)	13	12.5
Sudanese meat (SM)	5	4.81
Pastrami (P)	7	6.73
Frozen burger (B)	11	10.58
Frozen kofta (K)	15	14.42
Minced meat (MM)	6	5.77
Luncheon (LC)	19	18.27
Sausage (S)	7	6.73
Total bacterial number	104	100
p-value	<0.001	

Susceptibility test of different antibiotic drugs against selected bacterial isolates

The antibiotic susceptibility of the selected isolates to 10 antibiotics representing different groups showed that susceptibility of all isolated bacteria against different antibiotic drugs showed significant differences ($p<0.05$) except the susceptibility against Gentamicin excreted non-significant difference ($p>0.05$). In same context, the isolated bacteria were more resistant to Amoxicillin, Cefepime, Cotrimoxazole Cefuroxime, Cefatraixone, and Cefatoxime, whilst they were more susceptible to Ciprofloxacin, Vancomycinm and Tetracycline ($p<0.05$; Figure 1).

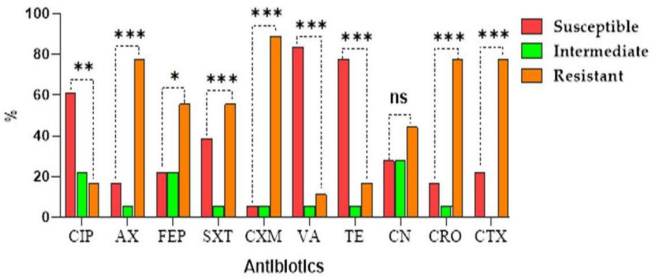

Fig. 1. Susceptibility of selected bacterial isolates against different antibiotic drugs. *p<0.05; **p<0.01;***p<0.001; ns non-significant

MIC and MBC

Results of minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations (µg/ml) for chosen drugs against selected bacterial isolates clearly indicated that there was a significant difference between isolated bacteria from frozen meat, frozen kofta, and luncheon with all studied drugs except Ciprofloxacin. Maximum values of MIC and MBC were detected in frozen meat isolates ($p<0.05$; Figure 2A-B).

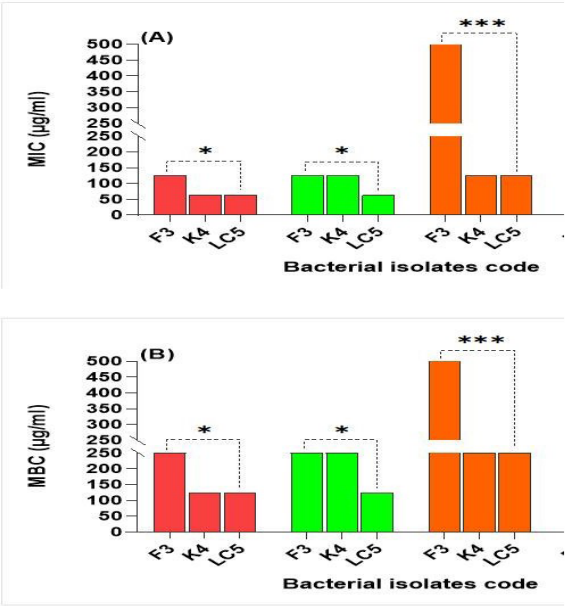

Fig. 2. Minimum inhibitory (A; MIC) and minimum bactericidal (B; MBC) concentrations (µg/ml) for chose drugs against selected bacterial isolates. *p<0.05; ***p<0.001; ns non-significant

Bacterial effects of different plant extracts against selected most resistant bacterial isolates

There are 12 plants (Basil, Garlic, Ginger, Rosella, Black Pepper, Zaater, Cumin, Turmeric Rosemary, Mint, Cinnamon and Clove) extracted aqueously by cold and hot water, and by methyl alcohol. The highest

mean of inhibition zone was detected with methanolic extract of clove as illustrated in Table 2.

MIC and MBC of clove extract

With respected to the minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations (%) of methanolic Clove extract against MDR bacteria. The present results showed significant differences in both MIC and MBC values maximized in luncheon isolated bacteria and minimized in frozen kofta, while they were intermediate in frozen meat ($p<0.05$; Table 3).

Purification of clove extracts principal compounds by using TLC

The separation of compounds on TLC revealed the presence of different compounds in clove extract. The different compounds purified in TLC were collected by scraping the different RF from TLC and evaluation of their antibacterial activity was carried out against clove extract. The promising compounds present in RF value (0.3) were further characterized and investigated by Gas Chromatography/Mass spectrometry (GC-MS).

Identification and purification of compounds in clove extract by using GC-MS

The characterization of compounds that are present in clove extract has revealed the presence of five antimicrobial compounds listed in Table 4 and Figure 3. These compounds have antimicrobial activity against tested pathogenic bacteria.

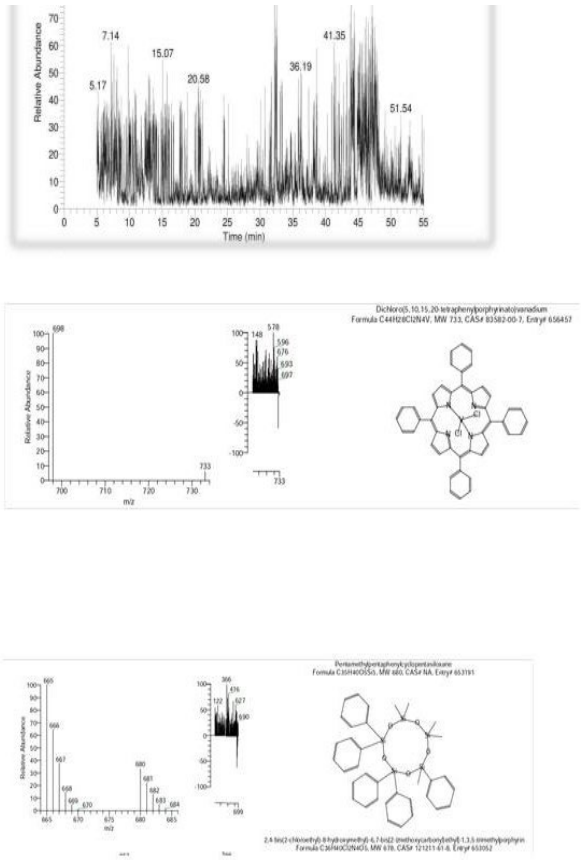

Fig. 3. Dimensional structures of active compounds present in the clove extract identified by GC-MS device.

Combination effect between antibiotics methanolic clove extract against selected MDR bacteria

Combination between antibiotics and methanolic clove extract

Table 2. Antibacterial effects of different plant extracts against selected most resistant bacterial isolates.

Bacterial isolates code		Diameter of Inhibition Zone (mm) of different plant extracts																													
		Basil			Garlic			Ginger			Rosella			Black Pepper			Zaater			Cumin			Turmeric			Rosemary			Mint			Cinnamon			Clove		
		Alc	H	C	Alc	H	C	Alc	H	c	Alc	H	C	Alc	H	C	Alc	H	C	Alc	H	C	Alc	H	C	Alc	H	C	Alc	H	C	Alc	H	C
F 3	0 (R)	2 (R)	4 (R)	4 (R)	25 (S)	24 (S)	26 (S)	9 (R)	8 (R)	4 (R)	10 (I)	16 (S)	17 (S)	11 (I)	1 (R)	0 (R)	9 (R)	8 (R)	7 (R)	12 (I)	10 (I)	9 (R)	9 (R)	0 (R)	0 (R)	14 (I)	3 (R)	5 (R)	1 (R)	0 (R)	1 (R)	15 (I)	12 (I)	11 (I)	17 (S)	10 (I)	8 (R)
K 4		10 (I)	5 (R)	4 (R)	12 (I)	13 (I)	0 (R)	7 (R)	4 (R)	0 (R)	14 (I)	15 I(I)	18 (S)	14 (I)	0 (R)	0 (R)	0 (R)	10 (I)	11 (I)	11 (I)	0 (R)	0 (R)	0 (R)	1 (R)	1 (R)	11 (I)	0 (R)	0 (R)	2 (R)	7 (R)	0 (R)	12 (I)	5 (R)	5 (R)	19 (S)	18 (S)	12 (I)
LC 5		4 (R)	6 (R)	7 (R)	1 (R)	20 (S)	17 (S)	18 (S)	0 (R)	1 (R)	16 (S)	12 (I)	11 (I)	11 (I)	0 (R)	1 (R)	3 (R)	0 (R)	2 (R)	0 (R)	11 (I)	13 (I)	0 (R)	0 (R)	0 (R)	19 (S)	2 (R)	0 (R)	0 (R)	1 (R)	0 (R)	3 (R)	0 (R)	0 (R)	22 (S)	15 (I)	11 (I)
B 2		2 (R)	5 (R)	9 (R)	0 (R)	15 (I)	16 (S)	17 (S)	2 (R)	3 (R)	14 (I)	17 (S)	15 (I)	11 (I)	0 (R)	0 (R)	28 (S)	4 (R)	0 (R)	11 (I)	0 (R)	10 (I)	11 (I)	7 (R)	6 (R)	9 (R)	1 (R)	0 (R)	10 (I)	0 (R)	0 (R)	4 (R)	0 (R)	0 (R)	12 (I)	11 (I)	10 (I)
LC 14		16 (S)	6 (R)	3 (R)	14 (I)	19 (S)	19 (S)	8 (R)	2 (R)	6 (R)	9 (R)	1 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	2 (R)	0 (R)	12 (I)	1 (R)	0 (R)	8 (R)	0 (R)	10 (I)	16 (S)	7 (R)	6 (R)	13 (I)	0 (R)	0 (R)	13 (I)	10 (I)	9 (R)	18 (S)	17 (S)	0 (R)
F 7		9 (R)	8 (R)	10 (I)	10 (I)	13 (I)	12 (I)	0 (R)	3 (R)	1 (R)	14 (I)	13 (I)	16 (S)	10 (I)	10 (I)	9 (R)	12 (I)	9 (R)	8 (R)	12 (I)	10 (I)	10 (I)	11 (I)	0 (R)	2 (R)	12 (I)	0 (R)	0 (R)	11 (I)	1 (R)	0 (R)	12 (I)	7 (R)	9 (R)	17 (S)	10 (I)	8 (R)
FS 4		11 (I)	10 (I)	10 (I)	17 (S)	19 (S)	16 (S)	1 (R)	1 (R)	3 (R)	15 (I)	17 (S)	16 (S)	2 (R)	4 (R)	0 (R)	0 (R)	0 (R)	0 (R)	10 (I)	0 (R)	8 (R)	7 (R)	0 (R)	1 (R)	0 (R)	2 (R)	1 (R)	12 (I)	2 (R)	0 (R)	15 (I)	3 (R)	2 (R)	16 (S)	4 (R)	1 (R)

ALC: Alcohol (methanol); H: Heat water; C: Cold water; R: Resistant; I: Intermediate; S: Susceptible

against selected MDR bacteria had significant effects on the inhibition zone diameter except the effects of Tetracycline, clove extract alone, Amoxicillin+ clove, Ciprofloxacin + clove as well as Amoxicillin+ Ciprofloxacin + clove showed non-significant effects ($p>0.05$). The inhibition zone diameter minimized for frozen meat, frozen kofta, and luncheon isolated bacteria treated by Tetracycline with non-significant differences ($p>0.05$; Table 5).

Table 3. Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations (%) of methanolic Clove extract against MDR bacteria.

Bacterial isolates code	Clove extract (%)	
	MIC	MBC
F 3	0.625[b]	1.25[b]
K 4	0.069[c]	0.625[c]
LC 5	1.25[a]	5.00[a]
p-value	<0.0001	<0.0001

[a,b,c]Means in the same column with different superscript letter following them are significantly different ($p < 0.05$).

Discussion

The present study was performed to determine the occurrence of bacterial pathogens. According to the results in Table 1, frozen meat (F) contains a high percentage of bacterial occurrences. This result is similar to that of Public Health England (2009); the allowable level of *Bacillus* group bacteria in food is below 10^3 cfu/g. However, doses as low as 10^3 *B. cereus* cfu/g of food sample may be sufficient to cause food poisoning (Gilbert and Kramer, 1986; Stenfors Arnesen *et al*., 2008).

In this study, commercially available and commonly used 10 antibiotics such as Amoxicillin, Gentamicin, Cefepime, Cotrimoxazole, Cefuroxime, Cefatraixone, and Cefatoxime, while they were more susceptible to Ciprofloxacin, Vancomycinm, and Tetracycline (Sigma, Saint. Louis, MO, USA), were selected for antibacterial susceptibility testing. Gentamicins are broad-spectrum antibiotics that inhibit the protein synthesis. The overall results of the disc diffusion assay revealed that all isolated pathogens showed statistically significant ($p<0.05$) sensitivity against Ofloxacin and Gentamicin, whereas they were resistant to Amoxicillin and Cefotaxime (Kiyomizu *et al*., 2009).

The MIC profiles showed that the majority of the antimicrobial agents evaluated in the study had modest levels of resistance. Nonetheless, a sizable level of resistance to all studied drugs except Ciprofloxacin was found. These outcomes are consistent with findings that indicated the use of several drugs (Jennifer, 2001).

Many types of secondary metabolites, including alkaloids, polyphenols, flavonoids, anthraguinones, coumarins, saponins, tannins, triterpenes, and steroids, were discovered through phytochemical screening. On pathogenic bacteria, several chemicals are active (Erfan and Marouf, 2019).

Investigation of clove extract show antimicrobial potency against the resistant bacteria, of the same by Sunil and Ammani (2009) who found that methanolic extract shows antibacterial activity against tested bacterial spp. and also confirmed by Mohamed *et al*. (2013) who recorded that the most active extracts observed by plants were against Gram positive and Gram negative bacteria. That activity may be due to the plant being rich in phytoconstituents like; tannins, phytosterols, sitosterols, saponins, anthroquinones, glycosides, and oleanolic compounds (Chen *et al*., 2005). The present results showed good inhibitory effects of clove extract on pathogenic bacteria. Many studies confirm the positive role of clove extracts in inhibiting pathogenic bacteria.

The antimicrobial potentiality of the above-mentioned plant methanolic extracts was determined by the standard agar disc diffusion technique, and the minimal inhibitory concentration (MIC) was determined in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2015) against the tested microorganisms was determined in accordance with Hannan, (2000).

The minimum inhibitory concentrations (MICs) for the extract and the antibiotics under study were determined in duplicate by the microbroth dilution method in Mueller-Hinton broth according to CLSI (Clinical Laboratory Standardization Institute) (Falagas *et al*., 2012).

The interactions between the extract and the antibiotics were determined using the checkerboard as previously described (Petersen *et al*., 2006).

The range of drug concentrations used in the checkerboard assay was such that the dilution range encompassed the MIC for each drug used in the analysis.

Phytochemical screening of the aqueous methanol fraction of clove revealed the presence of phytocompounds that have been documented to have antioxidant and other activities. Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen and various free radicals (Saeed *et al*., 2012), implicated in several diseases. Flavonoids have anti-oxidative and mucosal-protective effects (Sharath *et al*., 2015).

Table 4. The most potent antibacterial compounds identify by GC-MS device.

Compound name	Probability	RT	Area%	Molecular formula	Library
Dichloro (5,10,15,20-tet raphenylporphyrinato) vanadium	98.37	33.3	1.7	$C_{44}H_{28}CL_2N_4V$	Wiley
2-bis(ethoxycarbonyl)m ethyl-9 (2,3,5-tri-O-(2-m ethyl-prop-2-yl) dimethyl silyloxy-á-D-ribofuran osyl) purine	98.91	45.63	1.49	$C_{35}H_{64}N_4O_8Si_3$	Wiley
(2-Nitro-5,10,15,20-tet raphenyl[2-(2)H1]prop herniate) nickel (II)	95.62	32.42	2.3	$C_{44}H_{27}N_5NiO_2$	Wiley
(2-hydroxy-5,10,15,20-t etraphenylporphinato) zinc (II)	86.69	32.36	2.29	$C_{44}H_{28}N_4OZn$	Wiley
Pentamethylpentapheny lcyclopentasiloxane	63.4	20.06	1.68	$C_{35}H_{40}OSi_5$	Wiley

Table 5. Combination effect between antibiotics and methanolic clove extract against selected MDR bacteria

Bacterial isolates code	Antibiotic alone (MIC)				Clove extract alone	Combination between Methanolic clove extract with antibiotics							
	CRO	AX	TE	CIP		CRO	Effect	AX	Effect	TE	Effect	CIP	Effect
F 3	24[b]	18[a]	8	20[a]	19	25[a]	S	12	A	9[b]	S	16	A
K 4	32[a]	8[b]	7	10[c]	22	30[a]	A	11	S	12[ab]	S	11	S
LC 5	12[c]	7[b]	8	15[b]	21	13[b]	S	15	S	15[a]	S	14	A
p-value	<0.001	<0.001	0.83	0.00	0.53	0.00		0.06		0.05		0.23	

A: Antagonism; S: Synergism. [a,b,c] Means in the same column with different superscript letter following them are significantly different ($p < 0.05$).

Conclusion

Clove extract exhibited the highest bacteriostatic and bactericidal activities and can be used as a safe alternative to control microbial contamination of food.

Conflict of interest

The authors declare that they have no conflict of interest.

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