AUTHORS:
Kubrat A. Oyinlola[1]
Gbemisola E. Ogunleye[2]
Augustina I. Balogun[2]
Oluwarotimi Joseph[2]

AFFILIATIONS:
[1]Department of Microbiology, University of Ibadan, Ibadan, Nigeria
[2]Department of Biological Sciences, Koladaisi University, Ibadan, Nigeria

CORRESPONDENCE TO:
Kubrat Oyinlola

EMAIL:
bibimola.abiola@gmail.com

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Comparative study: Garlic, ginger and turmeric as natural antimicrobials and bioactives

Biologically active compounds in most spices possess antimicrobial and other important biomedical properties. There have been huge demands for natural immunity boosters (spices and herbs), considering the recent global pandemic and challenges relating to drug-resistant pathogens. This study was designed to compare the efficacy of ginger, garlic and turmeric spices against some pathogenic microorganisms. Aqueous extraction of spices, antimicrobial sensitivity and minimum inhibitory concentration tests were done using standard microbiological methods. Bioactive compounds were estimated using the gas chromatography–mass spectrometry (GC-MS) method. Aqueous extracts of ginger inhibited the growth of all test isolates except *Streptococcus pneumoniae*, with inhibition zones ranging between 0.9 mm and 13.5 mm. *Escherichia coli, S. pneumoniae* and *Haemophilus influenzae* were resistant to turmeric extracts, while the extract of garlic inhibited only four of the test pathogens. Inhibition zones for turmeric ranged between 4.4 mm and 10.9 mm, while those for garlic were between 4.7 mm and 11.5 mm. All the spice extracts did not inhibit microbial growth at 10–40%. An antibiotic spectrum indicated that *Bacillus* sp. was resistant to all but one, nitrofurantoin, which also inhibited the growth of almost all pathogens, except *H. influenzae*, with zones ranging between 10.5 mm and 11.6 mm. All test pathogens were resistant to cloxacillin except *E. coli* (10.6 mm). The major phyto-active compounds present in ginger are 2-Butanone,4-(4-hydroxy-3-methoxyphenyl), 1,3-Cyclohexadiene and 1-(4-Hydroxy-3-methoxyphenyl).

Significance:

Conclusively, ginger, turmeric and garlic have varied inhibitory activities against diverse organisms, indicating their antimicrobial properties; however, ginger showed a higher inhibitory effect and more diverse antimicrobial property amongst selected isolates. Furthermore, certain bioactive compounds of biomedical importance were present. We therefore recommend the use of these spices as alternative natural food preservatives against spoilage organisms, as well as potential natural sources for bioactive compounds in drug development against pathogens.

Introduction

Spice is an "aromatic vegetable substance in the whole, broken, or ground form, the significant function of which in food is seasoning rather than nutrition", according to the US Food and Drug Administration[1]. Globally, spice is used to enhance both the flavour and aroma of foods; however, their usage in preserving food quality and in the treatment of infections and diseases has been widely recognised[2], thus indicating their antimicrobial properties, as a result of certain naturally derived bioactive components[3]. Being organic and naturally plant based, spices are more widely accepted than synthetic additives like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), and, as such, their safety has not been questioned when compared. They have been widely employed in food processing and preparation, due to their antimicrobial properties that ensure improved food quality as well as prevent food spoilage.[4] Numerous phytochemical compounds in spices, such as isoflavones, anthocyanins, flavonoids, phenolic compounds, sulfur-containing compounds, tannins and alkaloids, acts as antimicrobial compounds and photoprotectants.[5] Early in the 20th century, the majority of the world's population depended on traditional preparations to address most medical conditions. Spices are regarded as safe and their efficacy against certain ailments has been recorded.[6] Various studies have revealed that oils and alkaloids in most spices possess antimicrobial, antiparasitic, immune booster, antioxidant and other important biological properties. Most recently, there has been huge demand for natural immunity boosters like spices and herbs, in response to the COVID-19 global pandemic.

However, the antimicrobial property spectrum of different spices varies[7], as it is difficult to predict the mechanism of microbial susceptibility. Different constituents may affect several targets, such as a microorganism's cell membrane, enzymes and/or their genetic material.[4,7] Research studies to tackle antibiotic drug resistance in microorganisms, through the quest for an alternative, have been widely prompted as some of these spices have shown great antimicrobial activities. The aim of this study was to evaluate the antimicrobial effect of the aqueous extracts of garlic (*Allium sativum*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) against some important foodborne and pathogenic microorganisms, as well as compare their activity with that of selected antibiotics and determine the presence of bioactive compounds.

Materials and methods

Sample collection

Fresh garlic (*Allium sativum*), turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) roots were locally sourced from a produce market in Ibadan, Oyo State, Nigeria. They were labelled and transported to the laboratory for immediate microbiological analysis.

Culture collection

Microorganisms used were obtained from the culture collection centre of the University College Hospital, Ibadan, and included *Escherichia coli, Candida albicans, Staphylococcus epidermidis, Staphylococcus aureus,*

Figure 1: Whole ginger, garlic and turmeric spices.

Pseudomonas aeruginosa, Haemophilus influenzae, Bacillus sp. *and Streptococcus pneumoniae.* The bacterial strains were resuscitated on nutrient agar (LabM, UK) before use.

Preparation of spice extracts

The modified method of Joe et al.[8] was used for the preparation of spices for extraction. The fresh spices were cleaned, peeled and subsequently washed in sterile distilled water (Figure 1). Samples were surface sterilised with 70% ethanol which was allowed to evaporate, after which the samples were rinsed with sterile distilled water. Samples (250 g of each) were cut into small sizes, crushed and blended, using a laboratory blender, to get a fine paste. The resulting mixture (of spices and sterile distilled water) was filtered through Whatman filter paper (No. 1) and sterilised using a membrane filter (0.45-μm filter unit, Merck). The filtrate was used as the 100% extract concentration. Appropriate volumes of sterile distilled water were mixed with the concentrate to obtain different concentrations (10%, 20%, 30% and 40%).

Antimicrobial sensitivity test

The agar well diffusion method was used[9] to test for the antimicrobial activity of the extracts. Test cultures were maintained on sterile nutrient broth for 18 h. They were further diluted out to 0.5 McFarland standard of approximately 1.5 x 10^8 CFU/mL. Cultures (0.1 mL) were aseptically inoculated on sterile Mueller–Hinton Agar (MHA; LabM, UK) plates and spread evenly, using a sterile cotton swab. Wells were made using a sterilised cork borer, and equal amounts (0.1 mL) of the different extracts (concentrated) were introduced into respective wells on the plates. Incubation was done in an upright position at 37 ºC for 24 h, and the diameter of inhibition zones was measured (Figure 2).

Antibiotic sensitivity test

Cultures were analysed using a modified Kirby-Bauer Disc Diffusion method for antibiotic sensitivity. Augmentin (amoxicillin/clavulanate, 30 μg), ofloxacin (5 μg), cloxacillin (5 μg), erythromycin (5 μg), ceftriaxone (30 μg), cefuroxime (5 μg), ceftazidime (30 μg) and nitrofurantoin (5 μg) were obtained from Hi-media, India. Aliquots (0.1 mL) of 18-hour-old cultures (0.5 McFarland) were aseptically swabbed on sterile MHA plates. Forceps, sterilised by flaming, were used to aseptically place the antibiotic discs over the seeded MHA plates. The discs were placed accordingly to prevent overlapping of expected inhibition zones. Incubation was done in an upright position at 37 ºC for 24 h, and the diameter of inhibition zones was measured.

Minimum inhibitory concentration

The broth dilution method[10] was used to determine the minimum inhibitory concentrations (MICs). Aliquots (0.5 mL) of each test organism (18-hour-old culture) were added to various concentrations (10%, 20%, 30% and 40% v/v) of the extracts, prepared by diluting with sterile distilled water, with a final volume of 5 mL. The cultures were incubated at 37 ºC for 24 h. Numbers of cells were determined using a spectrophotometer (Cecil CE 1011, Cambridge, UK) at 540 nm and were compared to the initial cell numbers.

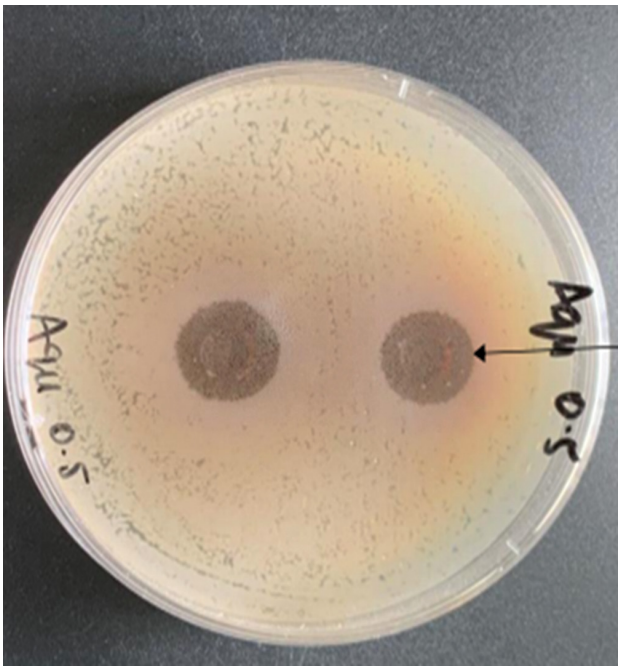

Figure 2: Representative plate showing zones of inhibition of the ginger aqueous extract against *Bacillus* sp.

Analysis of bioactive compounds

Identification and quantification of organic compounds in the extract with the most antimicrobial activity was done using gas chromatography–mass spectroscopy (GC-MS).[11]

Statistical analysis

Data obtained were subjected to statistical analysis (SPSS) at a 5% level of significance and are presented as the mean of replicates ± standard deviation.

Results

The results of the agar well diffusion test indicate that extracts of garlic, ginger and turmeric showed different degrees of growth inhibition, depending on the bacterial strain (Table 1). The ginger extract showed the broadest antibacterial activity by inhibiting the growth of all bacterial strains except one, with the diameters of inhibition zones ranging between 0.9 mm and 13.5 mm. The highest inhibition zone recorded (13.5 mm) was

Table 1: Antimicrobial activities of spice extracts (100%) against selected microorganisms

Isolate	Spices / diameter of inhibition zone (mean values in mm)		
	Turmeric	Ginger	Garlic
Escherichia coli	–	12.5	11.5
Bacillus sp.	4.4	12.1	–
Staphylococcus aureus	6.1	5.8	10.6
Staphylococcus epidermidis	2.3	5.1	–
Streptococcus pneumoniae	–	–	–
Candida sp.	10.3	12.6	10.2
Haemophilus influenzae	–	0.9	–
Pseudomonas aeruginosa	10.9	13.5	4.7

– No inhibition zone / resistant

against *P. aeruginosa,* while *S. aureus, S. epidermidis, S. pneumoniae, H. influenzae and Salmonella* sp. had inhibition zones that were less than 6 mm, indicating sensitivity of the ginger to the growth of these organisms.

Only three microbial isolates (*S. aureus, Candida* sp. and *P. aeruginosa*) showed inhibition sensitivity, indicated by inhibition zones greater than 6 mm, with the turmeric extract, with inhibition zones of 6.1 mm, 10.3 mm and 10.9 mm, respectively. However, the inhibition zones observed for the garlic extract ranged between 4.7 mm and 11.5 mm, the highest recorded against *E. coli. Bacillus* sp*., S. epidermidis, S. pneumonia* and *H. influenzae* were all resistant to the garlic extract. Overall, amongst the three spice extracts, ginger showed the highest inhibitory effect (13.5 mm), as well as a wide target range.

The sensitivity of different concentrations of the turmeric extract on the selected isolates is shown in Table 2. Against *E. coli*, the MIC of the extract was recorded at 10% with an optical density (OD) of 1.581 from an initial concentration of 1.642 OD at 540 nm. There was an increase in the OD of all other isolates, even when the concentration was increased from 10% to 40%.

The ginger extract, at 40%, was the MIC against *Bacillus* sp. with an OD of 1.499, while 30% was the MIC against *S. epidermidis, H. influenzae* and *P. aeruginosa,* with ODs at 540 nm of 1.490, 1.486 and 1.481, respectively. However, 10% was the MIC for the inhibition of *E. coli* (1.677). The concentrations used (10–40%) did not inhibit the growth of *S. aureus, S. pneumonia* and *C. albicans*. (Table 3).

Table 2: Effect of different concentrations of the turmeric extract on microbial growth

Test isolate	24-hour-old culture concentration	Extract concentration (%) / OD at 540 nm after incubation for 24 h			
		10	20	30	40
Escherichia coli	1.725	1.642 ± 0.00^{c}	1.933 ± 0.00^{a}	1.643 ± 0.00^{c}	1.681 ± 0.00^{b}
Bacillus sp.	1.525	1.915 ± 0.00^{a}	1.682 ± 0.00^{b}	1.670 ± 0.00^{c}	1.564 ± 0.00^{d}
Staphylococcus aureus	1.514	1.545 ± 0.00^{a}	1.540 ± 0.00^{b}	1.529 ± 0.00^{c}	1.525 ± 0.00^{c}
Staphylococcus epidermidis	1.501	1.550 ± 0.01^{a}	1.541 ± 0.00^{ab}	1.541 ± 0.01^{ab}	1.533 ± 0.00^{b}
Streptococcus pneumoniae	1.477	1.575 ± 0.00^{a}	1.573 ± 0.00^{a}	1.571 ± 0.01^{a}	1.505 ± 0.00^{b}
Candida sp.	1.440	1.732 ± 0.00^{a}	1.707 ± 0.00^{b}	1.619 ± 0.00^{d}	1.660 ± 0.00^{c}
Haemophilus influenzae	1.511	1.708 ± 0.00^{a}	1.670 ± 0.00^{c}	1.686 ± 0.00^{b}	1.648 ± 0.00^{d}
Pseudomonas aeruginosa	1.497	1.732 ± 0.02^{a}	1.699 ± 0.00^{b}	1.697 ± 0.01^{b}	1.689 ± 0.00^{c}

Values are mean ± standard deviation of three replicates

Means reported with the same superscript in each row indicate no significant difference ($p \leq 0.05$)

Table 3: Effect of different concentrations of the ginger extract on microbial growth

Test isolate	24-hour-old culture concentration	Extract concentration (%) / OD at 540 nm after incubation for 24 h			
		10	20	30	40
Escherichia coli	1.725	1.677 ± 0.00^{a}	1.645 ± 0.00^{b}	1.645 ± 0.01^{b}	1.488 ± 0.00^{c}
Bacillus sp.	1.525	1.661 ± 0.00^{b}	1.680 ± 0.00^{a}	1.661 ± 0.00^{b}	1.499 ± 0.00^{c}
Staphylococcus aureus	1.514	1.667 ± 0.00^{a}	1.561 ± 0.00^{b}	1.549 ± 0.00^{c}	1.533 ± 0.00^{d}
Staphylococcus epidermidis	1.501	1.575 ± 0.01^{a}	1.575 ± 0.01^{a}	1.490 ± 0.02^{b}	1.477 ± 0.00^{c}
Streptococcus pneumoniae	1.477	1.549 ± 0.00^{c}	1.627 ± 0.00^{a}	1.578 ± 0.00^{b}	1.481 ± 0.00^{d}
Candida sp.	1.440	1.670 ± 0.01^{a}	1.573 ± 0.00^{b}	1.573 ± 0.00^{b}	1.490 ± 0.00^{c}
Haemophilus influenzae	1.511	1.664 ± 0.01^{a}	1.516 ± 0.00^{b}	1.486 ± 0.00^{c}	1.479 ± 0.00^{c}
Pseudomonas aeruginosa	1.497	1.674 ± 0.00^{a}	1.510 ± 0.00^{b}	1.481 ± 0.00^{c}	1.360 ± 0.01^{d}

Values are mean ± standard deviation of three replicates

Means reported with the same superscript in each row indicate no significant difference ($p \leq 0.05$)

Table 4: Effect of different concentrations of the garlic extract on microbial growth

Test isolate	24-hour-old culture concentration	MIC (%) at 540 nm after incubation for 24 h			
		10	20	30	40
Escherichia coli	1.725	1.666 ± 0.00^{c}	1.782 ± 0.00^{a}	1.796 ± 0.00^{a}	1.747 ± 0.01^{b}
Bacillus sp.	1.525	1.661 ± 0.01^{d}	1.670 ± 0.00^{c}	1.816 ± 0.00^{a}	1.725 ± 0.00^{b}
Staphylococcus aureus	1.514	1.697 ± 0.00^{c}	1.854 ± 0.00^{a}	1.706 ± 0.00^{b}	1.654 ± 0.00^{d}
Staphylococcus epidermidis	1.501	1.781 ± 0.00^{b}	1.835 ± 0.00^{a}	1.728 ± 0.00^{c}	1.649 ± 0.01^{d}
Streptococcus pneumoniae	1.477	1.703 ± 0.00^{b}	1.778 ± 0.00^{a}	1.699 ± 0.01^{c}	1.660 ± 0.00^{d}
Candida sp.	1.440	1.826 ± 0.01^{a}	1.713 ± 0.00^{b}	1.674 ± 0.00^{c}	1.661 ± 0.00^{d}
Haemophilus influenzae	1.511	1.778 ± 0.00^{a}	1.747 ± 0.00^{b}	1.781 ± 0.00^{a}	1.646 ± 0.00^{c}
Pseudomonas aeruginosa	1.497	1.648 ± 0.01^{d}	1.848 ± 0.00^{a}	1.745 ± 0.00^{b}	1.682 ± 0.00^{c}

Values are mean ± standard deviation of three replicates

Means reported with the same superscript in each row indicate no significant difference ($p \leq 0.05$)

Table 5: Minimum inhibitory concentrations (MIC) for antimicrobial activity of spice extracts

Spice extract	MIC (%)							
	E. coli	*Bacillus* sp.	*S. aureus*	*S. epidermidis*	*S. pneumoniae*	*Candida* sp.	*H. influenzae*	*P. aeruginosa*
Ginger	10	40	–	40	–	–	40	40
Turmeric	10	–	–	–	–	–	–	–
Garlic	10	–	–	–	–	–	–	–

Table 6: Antibiotic susceptibility spectrum of selected isolates

Antibiotic	Test isolates / zone of inhibition (mm)							
	E. coli	*Bacillus* sp.	*S. aureus*	*S. epidermidis*	*S. pneumoniae*	*Candida* sp.	*H. influenzae*	*P. aeruginosa*
Augmentin	11.7	–	10.8	12.4	11.6	10.6	–	12.1
Ofloxacin	–	–	10.6	10.6	–	10.8	–	10.9
Cloxacillin	10.6	–	–	–	–	–	–	–
Erythromycin	10.2	–	10.6	–	–	10.7	13.1	10.5
Ceftriaxone	12.1	–	11.8	11.6	–	–	–	10.4
Cefuroxime	–	–	10.9	10.6	–	11.6	–	–
Ceftazidime	–	–	–	–	11.6	10.6	10.9	–
Nitrofurantoin	11.6	10.5	10.5	10.7	11.2	10.6	–	10.8

– No inhibition zone / resistant

Similar to the turmeric extract, only *E. coli* was inhibited at a 10% concentration of the garlic extract, with an OD of 1.666 at 540 nm. All other isolates were not inhibited by the extract concentrations (10–40%) used (Tables 4 and 5).

The antibiotic sensitivity pattern of selected isolates (Table 6) indicates that *Bacillus* sp. was resistant to all but one antibiotic (nitrofurantoin). *S. aureus* and *Candida* sp. were resistant to two of the tested antibiotics, cloxacillin for both, then ceftazidime and ceftriaxone, respectively. Nitrofurantoin, Augmentin and erythromycin showed sensitivity against a wide range of isolates; however, the highest inhibition zone (13.1 mm) was recorded for erythromycin against *H. influenzae*.

The ginger aqueous extract that showed the highest inhibitory effect was analysed for the phyto-active compounds present using GC-MS, and the results are presented in Figures 3–6. This extract generated 27 constituents, with major ones at peaks 18 (peak area 17.70%), 11 (peak area 13.30%) and 23 (peak area 10.84%) comprising 2-Butanone,4-(4-hydroxy-3-methoxyphenyl), 1,3-Cyclohexadiene and 1-(4-Hydroxy-3-methoxyphenyl), respectively.

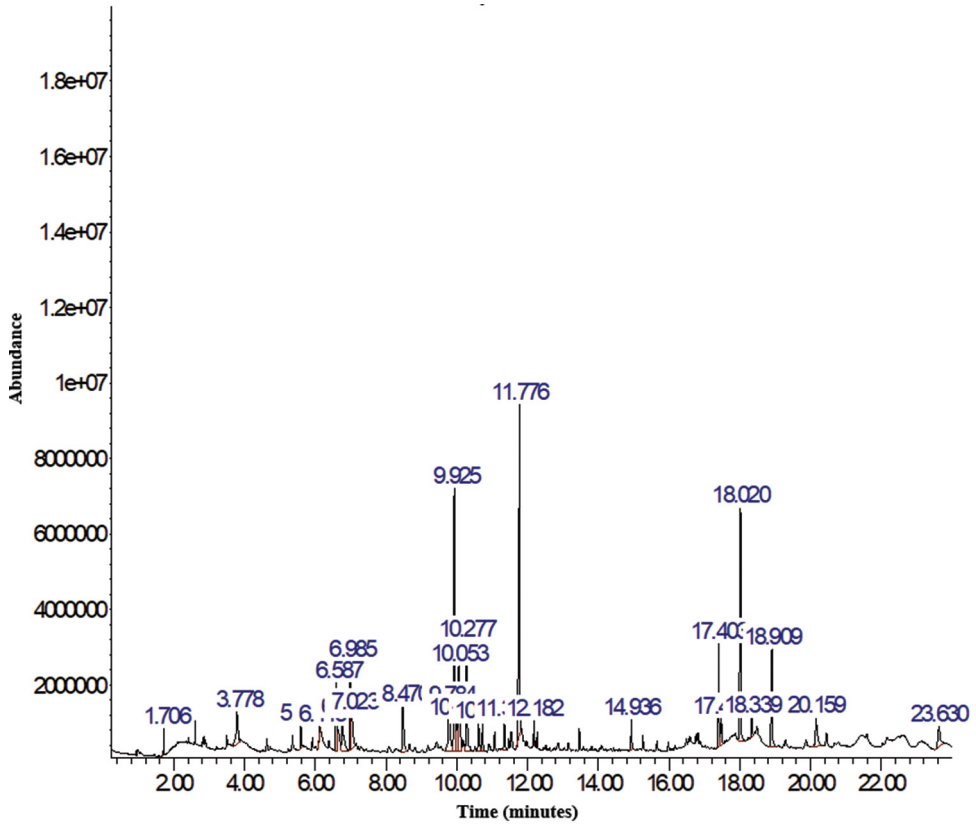

Figure 3: Total ion chromatogram of the ginger extract.

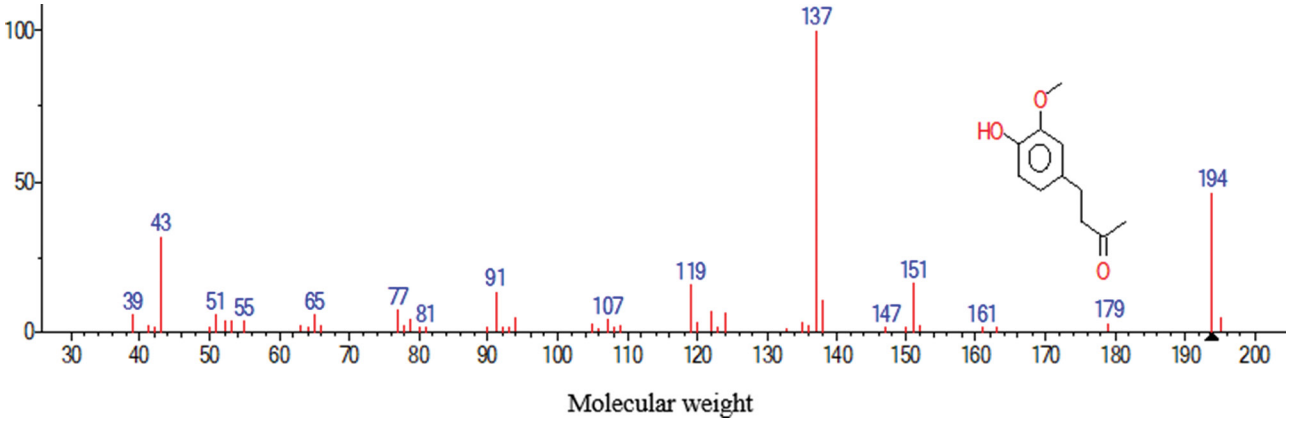

Figure 4: Chromatogram showing 2-Butanone,4-(4-hydroxy-3-methoxyphenyl) (% composition by area = 17.70; molecular weight 194).

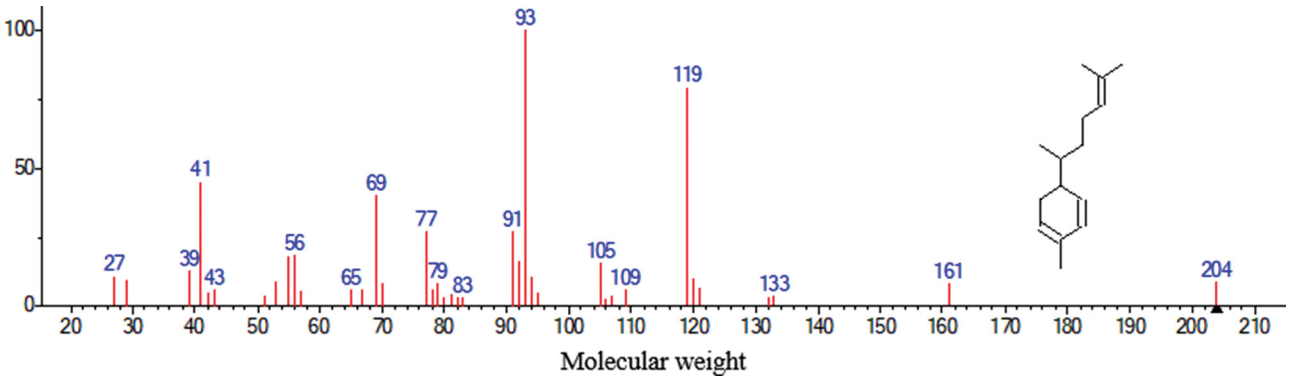

Figure 5: Chromatogram showing 1,3-Cyclohexadiene (% composition by area = 13.30; molecular weight 204).

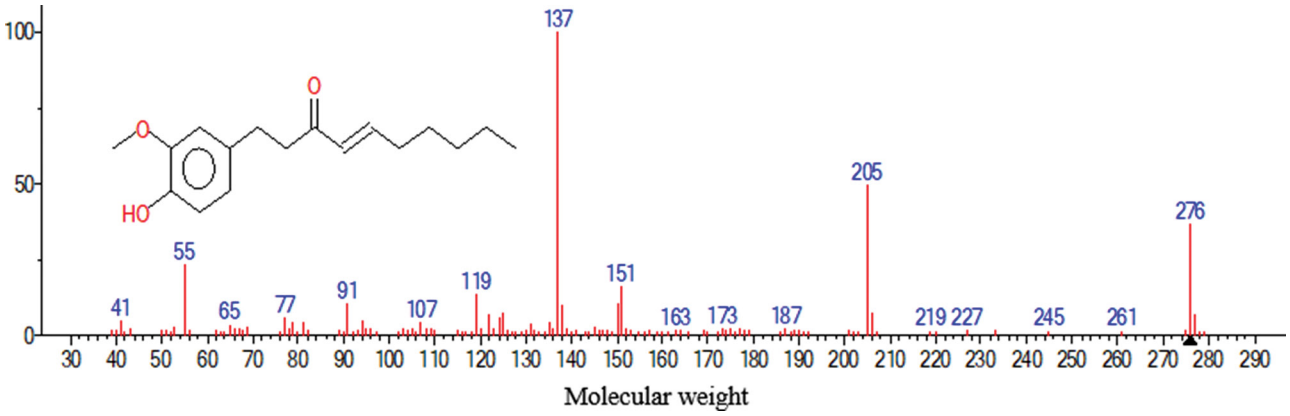

Figure 6: Chromatogram showing 1-(4-Hydroxy-3-methoxyphenyl) (% composition by area = 10.84; molecular weight 276).

Discussion

Use of spice and plant extracts has generally gained wide acceptability[11-14] because they are easy to use and highly efficient. Spices have beneficial biological functions such as bactericidal, bacteriostatic, fungistatic, anthelmintic, medicinal and flavouring properties.[15] In this study, not all the spice extracts showed inhibitory activity against all the test organisms. In contrast to our results which show that ginger exhibited the highest antimicrobial effect on test isolates, it was previously reported[13] that extracts of garlic had a strong effect against some pathogens, turmeric extracts exhibited a weak effect, and ginger extracts showed no inhibitory effect against any test bacteria during a study on the efficacy of spice extracts on bacterial isolates from meat products. It has been established that the antimicrobial activity of different spices may vary[7] towards different microorganisms, as the mechanism of microbial susceptibility is not predictable. The mechanism of action could be inhibition of cell wall synthesis, depolarisation of the cell membrane, inhibition of protein synthesis, inhibition of nuclei acid synthesis or inhibition of metabolic pathways.[16] The method followed may also result in such disparity, as reported in another study in which the antibacterial activity of spices was less evident when the paper disc method was used instead of the agar well assay method.[17]

Many studies have evaluated the antimicrobial activity of ginger, which has been shown to have a promising inhibitory effect against some pathogenic bacteria and fungi.[18-21] Ginger extracts were reported to have exerted an inhibitory effect against *Bacillus cereus, S. aureus and P. aeruginosa*.[14-21] In a study conducted using different spices (cinnamon, black pepper, cloves, turmeric and ajwain), antimicrobial zones of inhibition against *S. aureus* and *Klebsiella pneumoniae* ranged between 4 mm and 15 mm.[22] The maximum inhibition zone (13.5 mm) in the present study was within this reported range.

The mechanism of inhibitory action is dependent on the spices used. The major antimicrobial compound in garlic is allicin[23], while ginger has zingerone and gingerol. All have been reported to have strong inhibitory activities.[24] However, the general hypothesis is the integration of phenolic compounds to membrane proteins, thus leading to partitions in the lipid bilayer and effects on permeability, and subsequent membrane disruption.[25] The presence of lipophilic oils and hydrophilic antioxidants also contributes to good antibacterial activity.[26-28]

The minimum inhibitory concentration test against selected pathogens indicated a 10% MIC against *E. coli* for turmeric, garlic and ginger extracts. However, at 40%, ginger also inhibited the growth of a few other isolates, whereas most isolates were not inhibited by the garlic and turmeric concentrations used. Albaridi and Yehia[14] reported a 100 mg/mL concentration of ginger extract as the MIC against some selected pathogens. Different microorganisms responded differently to spice extracts at different concentrations.[8]

When comparing the inhibitory effect of these spices with those of different antibiotics, ginger particularly exhibited higher activity against *E. coli, Bacillus* sp., *Candida* sp. *and P. aeruginosa* than did the different antibiotics used. However, the inhibitory effect against *S. aureus, S. epidermidis, S. pneumoniae and H. influenzae* was more evident when antibiotics were used. Thus, ginger could be a potential source for drug development.

More than 60 bioactive constituents (volatile and nonvolatile compounds) are known to be present in ginger[29]; however, 2-Butanone,4-(4-hydroxy-3-methoxyphenyl), a phyto-active component of ginger, with a major peak area as revealed on the chromatogram, has been reported to have antioxidant, anti-inflammatory, anticancer and antimicrobial activities[30-33]. 1,3-Cyclohexadiene and 1-(4-Hydroxy-3-methoxyphenyl) have also been reported to have exhibited antimicrobial effects.[34] Ginger can thus be said to be of medicinal importance, rather than being highly and widely recognised only as a flavouring agent. It could be widely used in mitigating many human and other animal diseases.

Conclusion

Our findings demonstrate that different spices used have significant and varied activity against diverse organisms, indicating that natural products like ginger, turmeric and garlic have antimicrobial properties. Ginger showed the highest inhibitory effect against a wide range of isolates. A 10% minimum inhibitory concentration of all spices used inhibited the growth of *E. coli*, while concentrations up to 40% did not inhibit most isolates. Bioactive compounds of biomedical importance were present in the analysed ginger extract. Spices can be used as alternative natural food preservatives against spoilage organisms and as potential natural sources for drug development and use against pathogens.

Competing interests

We have no competing interests to declare.

Authors' contributions

K.A.O.: Conceptualisation; methodology; data collection; data analysis; writing the initial draft and revisions; project leadership. G.E.O.: Conceptualisation; methodology; data collection; validation; project management. A.I.B.: Sample analysis; methodology; student supervision. O.J.J.: Validation; data analysis; writing revisions.

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