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Antimicrobial activity of onion and ginger against two food borne pathogens *Escherichia coli* and *staphylococcus Aureus*

Abstract

Antimicrobial properties of phytochemicals extracted from onion (*Allium cepa*) and ginger (*Zingiber officinale*) were evaluated against *Escherichia coli* and Staphylococcus aureus, two common food borne pathogens. They were tested using minimum inhibitory concentration, minimum bactericidal concentration disc diffusion and agar wells diffusion. Onion showed good antimicrobial properties against *S.aureus* with an inhibition zone of 28mm but was not all that effective against *E.coli*. Ginger showed very poor antimicrobial properties against either of the organisms. *S.aureus* was shown to be more susceptible to the phytochemicals than *E.coli.*

Keywords: *Escherichia coli; Staphylococcus aureus;* Enterotoxins

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Objective and purpose

The purpose of this project was to investigate the antimicrobial effect of phytochemicals extracted from onion and ginger (fresh and boiled) at different concentrations, against *Escherichia coli* and *Staphylococcus aureus* using different methods (MIC, MBC, Disk and well diffusion). This project was chosen because onion and ginger are very common spices and have been claimed to contain several antimicrobial agents.

Hypothesis

i. *Escherichia coli* are more resistant to *Staphylococcus aureus* against chemical agents because it contains an outer membrane composed of lipopolysaccharide and protein which makes its membrane less permeable.

ii. Raw spice extract is more potent than the boiled spice extract.

Introduction

Food borne disease is gradually becoming a serious risk to public health with the number of cases increasing yearly.[1] The cause is determined to be from microorganisms, people become infected by either eating food infected with microbes or intoxicated with enterotoxins produced from microbes. Proper control of microbes and effective sanitation will help to reduce the rate at which people become sick from eating food. Spices in general show both antimicrobial and antioxidant characteristics, inhibiting the growth of bacteria and other pathogenic microbes.[1] Scientists in the past have conducted numerous experiments testing food borne pathogens with phytochemicals extracted from the spices.[2] The antimicrobial properties of active compounds extracted from spices and other plants in general based on recent findings have shown to affect the behavior of pathogenic bacteria and fungi in the agro food and medicinal sector.[1] This study investigates the antimicrobial activity of phytochemicals extracted from onion (*Allium cepa*) and ginger (*Zingiber officinale*) against *E.coli* and *S.aureus* using; Minimum inhibitory concentrations (MIC), Minimum bactericidal concentrations (MBC), and disc and agar well diffusion.

Onion and ginger

Onion has been revered throughout time not only for its culinary use, but also for its therapeutic properties. Consumption of onion is beneficial to human health as scientific studies show onion contains polyphenol molecules or phytonutrients which includes flavonoids, tannins as well as allicin which possess antioxidant and antimicrobial properties.[3] The main antimicrobial agent in onion isquercetin and allicin (thio-2-propene-1-sulfinic acid-5-allyl-esters), quercetin binds to the bacteria DNA gyrase while allicin inhibits certain thiol containing enzymes in the microorganisms by the rapid reaction of thiosulfinates.[4]

On the other hand, ginger has a long tradition of being very effective in alleviating symptoms of gastrointestinal distress.[3] Ginger can also be used as herbal medicine, and modern scientific research has revealed that ginger possesses numerous therapeutic properties that are similar to onion, including antioxidant effects, an ability to inhibit the formation of inflammatory compounds, and direct anti-inflammatory effects.[1] The main antimicrobial agent is gingerol a naturally occurring phenol which disrupts the cell wall of bacteria causing cytoplasmic leakage.[5]

Mininum inhibitory concentration and mininum bacteriacidal concentration

Minimum inhibitory concentrations (MIC) is the lowest concentration of any antibacterial substance that prevents or inhibits the growth of a particular pathogen after an overnight incubation period while minimum bacterial concentration (MBC) is the lowest antibacterial concentration that kills the pathogen after an overnight incubation period of about 24-48hours.[6] Minimum inhibitory concentrations (MIC) are regarded as the standard for determining the susceptibility of organisms to antimicrobials.[6]

Disc and agar well diffusion

The principle behind disc diffusion is relatively simple and easy to carry out, it involves an antibacterial impregnated disk placed on an agar previously inoculated with the test bacteria, when the disc picks up moisture the antibiotic diffuses outward through the agar producing an antibiotic concentration which produces zones of inhibition, the more susceptible the bacteria is to the antibiotic the greater the size of the zones.[7] The principle behind the agar well diffusion is similar to that of disc diffusion, wells uare bored into in the plate previously inoculated with the test bacteria and even volumes of the various antimicrobial agents are put into the wells which diffuse onto the agar and produces zones of inhibition.[7]

Escherichia coli

Escherichia coli is a Gram-negative, straight rod bacteria of about 1.1-1.5µm by 2.0-6.0µm occurring either singly or in pairs. They belong to the class *Proteobacteria*, the order *Enterobacteriales*, the family *Enterobacteriaceae* and the genus *Escherichia.*[7] They are motile by peritrichous flagella and facultatively anaerobic having both a respiratory and a fermentative type of metabolism (Bergey, 2000). Biochemically they are oxidase negative, catalase positive, methyl red positive, Voges-Proskauer negative and usually citrate negative.[8] *E.coli* occurs as normal flora in the lower part of the intestine, optimal growth for is about 37degrees but they are known to grow at temperatures of about 44.5degrees.[9] *E.coli* strains containing enterotoxins are known to cause diarrheal diseases due to their invasive and colonization characteristics, they are also a major cause of urinary tract infections and nosocomial infections including septicemia and meningitis.[9] When plated on differential media such as FC agar and Eosin methylene blue agar at 44.5 and 35degrees respectively for about 48hours they produce dark green metallic sheen colonies, when plated on MaCconkey agar at 35degrees they give pink colonies due to the fermentation of lactose.[10] *E.coli* was used for this study because it is a bacterial food borne pathogen responsible for infecting hundreds of people each year.

Staphylococcus aureus

Staphylococcus aureus is a gram-positive cocci bacterium that is a member of the Firmicutes group. It belongs to the class *Cocci*, order *Bacillales*, family *Staphylococcaceae* and genus *Staphylococcus*.[7] The cells are spherical about 0.5-1.5µm in diameter and in clusters or pairs. They are non-motile non-sporing facultatively anaerobic with both respiratory and fermentative mechanisms.[8] Colonies are usually white and cream on general purpose agar such as TSA or yellow on differential media such as MSA. Biochemically they are catalase positive and can grow in about 10% NaCl with an optimum temperature of about 30-37degrees.[9] They are mainly associated with the skin and mucus membrane of warm blooded animal and serves as an indicator of nasal contamination in foods. They are known as opportunistic pathogen in humans causing diarrhea by producing extracellular toxins.[9] *S.aureus* is an important food borne pathogen causing several incidents of food poisoning which leads to diarrhea.

Media

MacConkey agar: MacConkey Agar is used for the detection and enumeration of gram negative bacteria based on their ability to ferment lactose. Bile salts and crystal violent serves as the selective agent while phenol red is the indictor. *E.coli* a lactose fermenter grows extremely well on MAC producing pink colonies with red zones.[10]

MR-VP BROTH: MR-VP Medium and MR-VP Broth (Methyl Red-Voges Proskauer Medium/Broth, are used for the differentiation of bacteria by means of the methyl red and Voges-Proskauer reactions.[11]

Simmons citrate agar: Simmons Citrate Agar is used from differentiating bacteria which can utilize citrate as the sole source of carbon and inorganic ammonium salt as the sole source of *Escherichia coli* either do not grow at all on this medium, or grow so sparsely that no change in reaction is apparent as the media still remains green. Sodium Citrate is the sole source of carbon in this medium. Magnesium Sulphate is a cofactor for a variety of metabolic reactions. Bromthymol Blue is the pH indicator.[12]

Mannitol salt agar: Mannitol Salt Agar is highly selective and used to isolate coagulase positive *Staphylococcus aureus* by inhibiting growth of most other bacteria with a high salt concentration of about 7.5% Sodium Chloride. Bacteria that grow in the presence of a high salt concentration and ferment mannitol to produce acid products, turning the Phenol Red pH indicator from red to yellow. *Staphylococcus aureus* ferment mannitol and form yellow colonies with yellow zones. (Neogen 2015).

Mueller hinton broth and agar: Mueller Hinton Broth and Agar are used for performing antibiotic susceptibility tests (MIC, MBC) using a single disk of high concentration. The medium provides excellent growth for most non-fastidious pathogens due to its low concentration of sulphonamide.[13]

Tryptic Soy Broth: Tryptic Soy Broth is a general purpose medium. Tryptic Soy Broth is used for the preparation of inoculum used in antibiotic tests. Casein and Soybean Meal serves as the source of nitrogen in TSB. Dextrose is the carbon source which enables the organism to grow. Osmotic balance and buffering agent are maintained by Sodium Chloride and Dipotassium Phosphate. Most clinical tested microbes grow extremely on TSB.[14]

Recent research conducted on the antimicrobial effect of ginger and onion showed weak to medium antibacterial properties.[1] The author used agar well diffusion to determine the zones of inhibition. Also results according to Onyeagba et al.[15] also showed both ginger and onion to have significant antimicrobial properties.

Materials

Bacteria

i. *Escherichia coli* ATCC 25922 obtained from Prep Room.

ii. *Staphylococcus aureus* ATCC 25923 obtained from Prep Room.

Spices

i. Onion (*Allium cepa*) obtained from Food Basic along Neilson Road.

ii. Ginger (*Zingiber officinale*) obtained from Food Basic along Neilson Road.

Media

i. MacConkey Agar

ii. Mannitol Salt Agar

iii. Mueller Hinton Agar

iv. Simmon Citrate Agar (SIM)

v. Tryptic Soy Broth

vi. Mueller Hinton Broth

vii. MR-VP Broth

viii. Coagulase

Supplies and equipment

i. Blender

ii. Hot plate

iii. Chopping Board and Knife.

iv. Mortar, Pestle and cheese cloth

v. Antimicrobial Disks

vi. Ampicillin disks obtained from the prep room

vii. Ciprofloxacin disks obtained from the prep room

viii. Hydrogen peroxide for Catalase test

ix. Phosphate

Methods

Sample collection

100grams of onion and 100grams of ginger were purchased from Food basic store along Neilson road.

Restreaking on selective media

A colony of *E.coli* obtained from the prep room was streaked on a MaCconkey agar plate and incubated at 35degrees for 48hours; the same was done with a colony of *S.aureus* on an MSA plate and incubated at 35degrees for 48hours. Restreaking on selective was carried out regularly at the beginning of every week in other to have a fresh supply of bacterial colonies needed for the experiment. It was used for bacteriological analysis and biochemical characterization.

Morphological Characterization

The isolated microbes from both Mac cultures and MSA cultures were characterised morphologically on the basis of simple gram staining.

Biochemical characterization

The isolates were characterised by biochemical tests using citrate utilization test, Methyl red test and Voges Proskauer test for *E.coli* and catalase and coagulase test for *S.aureus*, this was carried out in order to determine purity of tested micro-organism. A single colony of *E.coli* was inoculated on citrate agar slant and MR-VP broth for 48hours and incubated at 35degrees after which two drops of MR reagent and VP reagents were added. Also, a colony of *S.aureus* was placed on a microscope slide and three drops of hydrogen peroxide was added onto it and viewed for the formation of gas bubbles which indicates the presence of catalase. Coagulase test was also carried out on the same colony of *S.aureus,* which was inoculated and incubated for 48hours at 35degrees. Negative controls were also carried out by incubating the media used without any inoculate for 48hours at 35degrees.

Preparation of Inoculate

Isolated colonies of *E.coli* and *S.aureus* from the Mac and MSA cultures were inoculated in a test tube containing 9ml of TSB and incubated at 35degrees for 48hours. The overnight cultures obtained from the tubes were used to determine the minimum inhibitory concentration of onion and ginger extracts.

Preparation of onion and ginger extracts

The onion and ginger obtained, was cleaned and washed using sterile distilled water. Using a disinfected knife and chopping board the samples were cut into smaller pieces, crushed in a mortar using a pestle and liquefied in a blender. The extracts were then sieved through a sterile cheese cloth into a sterile Erlenmeyer flask; this extract was considered as the 100% raw extract. The same procedure was repeated and the extract was boiled in a sterile conical flask for 5minutes, this was thus considered as the 100% boiled extract.

Determination of Minimum Inhibitory Concentration (MIC)

0ml, 1ml, 2ml, 3ml, 4ml, and 5ml of Mueller Hilton broth was pipetted into different sterile test tubes. After which 10ml, 9ml, 8ml.7ml, 6ml and 5ml of each extract was added into the corresponding tubes containing 0ml, 1ml, 2ml, 3ml, 4ml and 5ml of Mueller Hinton broth. This represented 100%, 90%, 80%, 70%, 60% and 50% concentration of each extract. Using a sterile Pasteur pipette 2drops of each culture was added into each tube respectively. This was done for both the raw and boiled extract for both onion and ginger for each organism and thus a total of 48 tubes was incubated at 35degrees for 48hours and observed for growth by turbidity.

Negative controls were also set up using 5ml of both the raw and boiled extract for onion and ginger and incubated at 35degrees for 48hours. Media controls were also set up using 10ml of Mueller Hinton broth and incubated at 35degrees for 48hours. The purpose of the control was to determine the presence of growth and no growth, to determine if the media was contaminated and to eliminate false positives.

Determination of Minimum Bactericidal Concentration (MBC)

A loop-full from each tube containing the different concentrations of both onion and ginger extract with the respective microbes was plated onto a plate containing Mueller Hilton agar. Each tube was plated onto the same plate with different partitions indicating concentration. In all a total of 8 plates one for each microbe and spice (boiled and raw) was incubated for 48 hours at 35 degrees and observed for growth on the surface of the agar.

Negative controls were also done by incubating an empty plate of Mueller Hinton agar for 48 hours at 35 degrees. This was done to check sterility of media.

Disc diffusion and agar well diffusion

Each tube containing overnight cultures of *E.coli* and *S.aureus* were diluted in phosphate buffer to a concentration of 2.5Mcfarland. After which 0.1ml of each culture was spread across the surface of the plates containing MH agar. 24sterile discs were used for each organism and each spice which was impregnated in various concentrations ranging from 100-50% and placed on the surface of the agar which

was incubated for 35degrees at 48hours and examined for zones of inhibition. Inhibition zones less than or equal to 7mm were considered to have no antimicrobial effect.

Positive controls were also set up using ampicillin and ciprofloxacin disc which was placed onto the surface of the agar and incubated for 48hours at 35degrees. Media controls were also set up by incubating a plate of MH agar for 48hours at 35degrees. Agar well diffusion followed the same principle as disc diffusion the only difference been well were bored in the agar already inoculated with the test microbes and different concentrations of spice extracts were placed into the wells which was incubated for 48hours and 35degrees.

Results

Biochemical characterisation

The following tables show the biochemical characterisation of the two isolates used. Simmon citrate agar and MR-VR broth were used for *E.coli*, inoculated and were incubated for 48hours at 35degrees, while the coagulase test was used for *S.aureus* and was also incubated for 48hours at 35degrees. The catalase test was also carried out on *S.aureus* (Table 1).

Citrate utilization test showed *E.coli* does not utilize citrate due to the media remaining unchanged this is a typical *E.coli* reaction. When the MR reagent was added a bright red color appeared this is regarded as a positive result while when the VP reaction was added there was no change, this is regarded as a negative result which is typical to *E.coli* (Table 2).

Coagulase test was positive due to clot which showed *S.aureus* produces coagulase and the presence of gas bubbles also indicated that the catalase test was positive.

Morphological Characterisation

The following table shows the morphological characterisation and gram reaction of *E.coli* and *S.aureus* (Table 3). Morphological characterisation of *E.coli* was characterised by the presence of red rod shaped cells in random order and purple cocci shaped cells in clusters for *S.aureus* with indicates a typical morphological characterisation for both cells.

Minimum inhibitory concentration

The following tables show the result for the Minimum Inhibitory Concentration of both the raw and boiled extract of both species which was diluted in Mueller Hinton broth to get specific concentrations, inoculated with overnight culture of *E.coli* and *S.aureus* and incubated for 48hours at 35degrees (Table 4).

Due to the nature of the extracts the negative controls were used to determine the presence and absence of growth by comparing it with the inoculated tubes. The Negative controls showed no growth in them (Table 5).

The presence of growth was identified by the turbidity of the tubes and the presence of a whitish pellicle on the surface of the tubes. Also the presence of no growth was identified by the similarity of the tubes to the negative controls (Table 6).

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Minimum bactericidal concentration

The following tables show the result for the minimum bactericidal concentration of all tubes inoculated, a loopful was transferred unto partitioned sections on a plate of Mueller Hilton agar and incubated for 35degrees at 48hours (Table 10).

MBC results showed that 80% concentration of raw onion was capable of killing the *E.coli* cells. The ginger extract on the other hand was not capable of killing the cells (Table 11).

MBC results showed that 70% concentration of raw onion was capable of killing the *S.aureus* cells. The ginger extract on the other hand was not capable of killing the cells. MBC for boiled onion and ginger extract (Table 12). MBC results showed both the onion and ginger extract was not capable of killing the cells (Table 13).

Disc diffusion

The following tables shows the results for the antimicrobial activity of the spice extract against *E.coli* and *S.aureus* measured as the diameter of growth inhibition using impregnated discs with different concentration of the spice extracts which were diluted in different volumes of distilled sterile water. Positive controls were also carried out using ampicillin and ciprofloxacin disc on inoculated plates of *E.coli* and *S.aureus* incubated for 48 hours at 35 degrees (Table 14) & (Table 15). The result showed onion to have good antimicrobial properties *S.aureus* but also showed ginger having no antimicrobial properties against *S.aureus* (Table 16). The result showed boiled spice extract to have no antimicrobial properties against both organisms (Table 17).

Agar well diffusion

The following tables shows the results for the antimicrobial activity of the spice extract against *E.coli* and *S.aureus* measured as the diameter of growth inhibition from the wells bored in the agar, each well contained a different concentrations of spice extracts. The plates were incubated for 48hours at 35degrees. The diameter of the wells were 7mm (Table 18). The zones of inhibition gotten above showed both onion and ginger to have no antimicrobial activity on *E.coli* (Table 19). The result showed onion to have fairly good antimicrobial properties *S.aureus* but also showed ginger having no antimicrobial properties against *S.aureus*.

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Table 1 Biochemical characterisation results of *E. coli* using Simmon citrate agar to test for citrate utilization and MR-VR broth inoculated and incubated 48hours at 35degrees

Biochemical tests	Citrate test	Mr reagent	Vp reagent
Reactions	Green medium	Bright red (positive)	No red color (Negative)

Table 2 Biochemical characterisation results of S. aureus using coagulase which was inoculated and incubated for 48hours at 35degrees and catalase test using Hydrogen Peroxide

Biochemical tests	Coagulase	Catalase
Reactions	Clot formation	Bubbles

Table 3 Morphological characterisation of E. coli and S. aureus using simple gram staining and viewed under a microscope at

Bacteria	*E. coli*	*S. aureus*
Gram reactions & morphology	Negative, Rod shaped in a random order	Positive Cocci in clusters

Table 4 Negative controls of raw extracts from onion and ginger which was incubated at 35degrees for 48hours

Controls	Onion	Ginger
Description	Slightly clear with particles settled at the bottom	Yellowish slightly clear solution with particles settled at the bottom.

Table 5 MIC of various concentrations of raw onion and ginger extract against inoculated strains of E. coli which was incubated at 35degrees for 48hours

Onion conc	100%	90%	80%	70%	60%	50%
Observation of Tubes	Slightly clear with particles settled at the bottom	Slightly clear with particles settled at the bottom	Slightly clear with particles settled at the bottom	Slightly clear with particles settled at the bottom	Slightly clear with particles settled at the bottom	Turbid with particles settled at the bottom
Ginger conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Observation of Tubes	Whitish pellicle on the surface of the tubes.	Whitish pellicle on the surface of the tubes.	Whitish pellicle on the surface of the tubes.	Whitish pellicle on the surface of the tubes.	Whitish pellicle on the surface of the tubes.	Whitish pellicle on the surface of the tubes.

Table 6 MIC of various concentrations of raw onion and ginger extract against inoculated strains of S. aureus which was incubated at 35degrees for 48hours.

Onion conc	100%	90%	80%	70%	60%	50%
Observation of tubes	Slightly clear with particles settled at the bottom	Slightly clear with particles settled at the bottom	Slightly clear with particles settled at the bottom	Slightly clear with particles settled at the bottom	Slightly clear with particles settled at the bottom	Turbid with particles settled at the bottom
Ginger conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Observation of tubes	Yellow liquid with particles settled at the bottom	Yellow liquid with particles settled at the bottom.	Whitish pellicle on the surface of the tubes.	Whitish pellicle on the surface of the tubes.	Whitish pellicle on the surface of the tubes	Whitish pellicle on the surface of the tubes.

Table 7 Negative controls of boiled extracts from onion and ginger which was incubated at 35degrees for 48hours

Controls	Onion	Ginger
Description	Slightly clear with particles settled at the bottom	Yellowish slightly clear solution with particles settled at the bottom.

Table 8 MIC of various concentrations of boiled onion and ginger extract against inoculated strains of E. coli which was incubated at 35degrees for 48hours

Onion conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Observation of Tubes	Turbid with particles settled at the bottom	Turbid with particles settled at the bottom	Turbid with particles settled at the bottom	Turbid with particles settled at the bottom	Turbid with particles settled at the bottom	Turbid with particles settled at the bottom
Ginger conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Observation of Tubes	Whitish pellicle on the surface of the tubes	Whitish pellicle on the surface of the tubes	Whitish pellicle on the surface of the tubes	Whitish pellicle on the surface of the tubes	Whitish pellicle on the surface of the tubes	Whitish pellicle on the surface of the tubes

Table 9 MIC of various concentrations of boiled onion and ginger extract against inoculated strains of S. aureus which was incubated at 35degrees for 48hours

Onion conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Observation of Tubes	Turbid with particles settled at the bottom	Turbid with particles settled at the bottom	Turbid with particles settled at the bottom	Turbid with particles settled at the bottom	Turbid with particles settled at the bottom	Turbid with particles settled at the bottom
Ginger conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Observation of Tubes	Whitish pellicle on the surface of the tubes	Whitish pellicle on the surface of the tubes	Whitish pellicle on the surface of the tubes	Whitish pellicle on the surface of the tubes	Whitish pellicle on the surface of the tubes	Whitish pellicle on the surface of the tubes

Table 10 MBC results of the various concentration of raw onion and ginger extract against E. coli, inoculated and incubated on Mueller Hilton agar for 35degrees at 48hours

Onion conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Growth/no growth	No growth	No growth	Growth	Growth	Growth	Growth
Ginger conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Growth/no growth	Growth	Growth	Growth	Growth	Growth	Growth

Table 11 MBC results of the various concentration of raw onion and ginger extract against S. aureus, inoculated and incubated on Mueller Hilton agar for 35degrees at 48hours

Onion conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Growth/no growth	No growth	No growth	No growth	No growth	Growth	Growth
Ginger conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Growth/no growth	Growth	Growth	Growth	Growth	Growth	Growth

Table 12 MBC results of the various concentration of boiled onion and ginger extract against S. aureus, inoculated and incubated on Mueller Hilton agar for 35degrees at 48hours

Onion conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Growth/no growth	Growth	Growth	Growth	Growth	Growth	Growth
Ginger conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Growth/no growth	Growth	Growth	Growth	Growth	Growth	Growth

Table 13 MBC results of the various concentration of boiled onion and ginger extract against E. coli, inoculated and incubated on Mueller Hilton agar for 35degrees at 48hours

Onion conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Growth/no growth	Growth	Growth	Growth	Growth	Growth	Growth
Ginger conc	100%	90%	80%	70%	60%	50%
Growth/no growth	Growth	Growth	Growth	Growth	Growth	Growth

Table 14 Positive control using ampicillin and ciprofloxacin disc on plates containing E. coli and S. aureus incubated for 48hours at 35degrees

Ampicillin(*E. coli*)	**Ciprofloxacin (*E. coli*)**
Zone: 21mm	Zone: 45mm
Ampicillin (*S. aureus*)	**Ciprofloxacin (*S. aureus*)**
Zone: 46mm	Zone: 30mm.

The diameter of the disc was 7mm.

Table 14 Positive control using ampicillin and ciprofloxacin disc on plates containing E. coli and S. aureus incubated for 48hours at 35degrees

Ampicillin(*E. coli*)	**Ciprofloxacin (*E. coli*)**
Zone: 21mm	Zone: 45mm
Ampicillin (*S. aureus*)	**Ciprofloxacin (*S. aureus*)**
Zone: 46mm	Zone: 30mm.

Table 15 Disc diffusion results for various concentrations of raw onion and ginger extract against E. coli on Mueller Hinton agar and incubated for 48hours and 35degrees

Onion Conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Size of zone	7mm	7mm	7mm	7mm	7mm	7mm
Ginger Conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Size of zone	7mm	7mm	7mm	7mm	7mm	7mm

The zones of inhibition gotten above showed both onion and ginger to have no antimicrobial activity on E. coli.

Table 16 Disc diffusion results for various concentration of onion and ginger extract against a spread of S. aureus on Mueller Hilton Agar and incubated for 48hours and 35degrees

Onion conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Size of zone	28mm	27mm	25mm	24mm	23mm	20mm
Ginger conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Size of zone	7mm	7mm	7mm	7mm	7mm	7mm

Table 17 Disc diffusion results for boiled onion and ginger extract against a spread of S. aureus and E. coli incubated for 48hours at 35degrees

E. coli	*S. aureus*
Onion 100%	**Onion 100%**
Zone :7mm	Zone :7mm
Ginger 100%	**Ginger 100%**
Zone: 7mm	Zone: 7mm

Table 18 Agar well diffusion results for various concentration of onion and ginger extract against a spread of E. coli on Mueller Hilton agar and incubated for 48hours and 35degrees

Onion Conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Size of zone	7mm	7mm	7mm	7mm	7mm	7mm
Ginger Conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Size of zone	7mm	7mm	7mm	7mm	7mm	7mm

Table 19 Agar well diffusion results for various concentration of onion and ginger extract against a spread of S. aureus on Mueller Hilton agar and incubated for 48hours and 35degrees

Onion Conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Size of zone	15mm	14mm	13mm	10mm	7mm	7mm
Ginger Conc	**100%**	**90%**	**80%**	**70%**	**60%**	**50%**
Size of zone	7mm	7mm	7mm	7mm	7mm	7mm

Discussion

Natural products including spices have been used for years because of their therapeutic and antimicrobial properties. The purpose of this project was to investigate the antimicrobial properties of two of such spices, onion and ginger.

The experiment was conducted in two stages, the preliminary and final stage, the result for the former were not recorded in the previous chapter due to irregularities and numerous errors resulting from improper aseptic technique during the extraction of the spices which lead to cross contamination and unreasonable results.

The result of this experiment showed *Staphylococcus aureus* to be more susceptible to *Escherichia coli,* which makes *E.coli* more resistant to the spice extracts, thus confirming the first hypothesis stated earlier. Results were obtained using three techniques (MIC,MBC, Agar diffusion) based on this ginger showed little to no antimicrobial activity against either organism particularly *E.coli*, this was similar to the results gotten by Serthi et al.[1]

Based on the results, the raw onion extract showed a greater antimicrobial activity against *S.aureus* with an MBC concentration of 70% v/v and an inhibition zone of 28mm been the largest. The activity on *E.coli* was different as no zones of inhibition were gotten which was similar to results gotten by Onyeaba et al.[15] 90% v/v concentration was shown to kill *E.coli*, this was unusual as the same concentration showed no zone of inhibition when re-evaluated using disc diffusion. This could be as a result of two possible lab errors, either the bacterial lawn was too thick which rendered the extract inactive or no cells were transferred from the MIC tube to the MBC plates.

In addition, the boiled spice extract showed no antimicrobial activity against either organism thus confirming the second hypothesis. This could be as a result of the high temperature which must have deactivated the active agents rendering them ineffective against both bacteria. Due to the nature of the spice extracts, the broth dilution technique was not ideal in evaluating MIC as it was difficult to identify the presence and absence of growth, this was shown extensively during the preliminary stage. As such other techniques such as agar dilution and Epislometer test should be used when dealing with the same or similar spices.

It is also important to note that the experiment was affected by various factors which include concentration of spice extracts, volume of agar, and concentration of culture and incubation times. Future studies should be conducted in a more controlled environment were these factors are constant. Also future research should observe if the phytochemicals present in spices showed synergetic or antagonistic

properties against each other. In conclusion, each particularly phytochemical should be extracted, purified and tested individually for its antimicrobial properties.

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Conflict of interest

The author declares no conflict of interest.

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