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Effects of Phytochemicals and antioxidants of indigenous plant spices against bacterial isolates from pork meat sold in Owerri, Imo State, Nigeria

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Abstract

This study is to determine the phytochemical and antioxidant properties of plant spices; *Capsicum frutescens* (Ghana Pepper), *Piper guineense* (*Uziza*), *Ocimum gratissimum* (Scent Leaf), *Allium cepa* (Onions)and *Gongronema latifolium* (*Utazi*) against food-borne bacterial isolates from pork meat in Owerri. All analysis was done using standard methods. In this study, some bacteria were implicated in the pork meat sold by different vendors in various locations (Obinze, Ihiagwa, Umuchima, FUTO Backgate and Eziobodo). Phytochemicals were synthesized from the spices and shown to demonstrate antimicrobial properties. The heterotrophic bacterial count and the zones of inhibitions of the plant spices against the food borne bacterial isolates were determined using plate count techniques and disc diffusion respectively. The percentage occurrences of these bacteria are listed thus: *Salmonella* sp (6.42%), *Escherichia coli* (17.44%), *Shigella* sp (6.17%), *Staphylococcus* sp (7.05%), *Bacillus cereus* (4.72%), *Bacillus subtilis* (5.92%), *Enterococcus* sp (4.79%), *Staphylococcus aureus* (5.16%), *Vibrio cholerae* (18.14%), *Pseudomonas* sp (7.18%) and *Vibrio parahaemolyticus* (17.00%). All plant samples contain phytochemicals and exhibited antioxidant properties. In a decreasing order, the phytochemicals are as follows; Saponins > Terpenoids > Tannins > Flavonoids > Steroids > Alkaloids > Glycosides > Anthraquinone. *Allium cepa* has the highest level of phytochemicals while *Ocimum gratissimum* possesses the least phytochemical property. The concentration of these phytochemicals ranges from (0.13±0.01) – (8.71±0.01). All plant spices in this study exerted antioxidant properties. In a decreasing order in relation to their antioxidant properties, the plant spices are as follows; *Allium cepa* (83.80%±0.04) >*Capsicum frutescens* (73.22%±0.03) >*Ocimum gratissimum* (65.42%±0.02) >*Gongronema latifolium* (62.03%±0.02) >*Piper guineense* (31.84%±0.01). These findings demonstrate antibacterial efficiencies of the plant spices against food-borne bacterial isolates from pork meat and indicate that these spices may be useful therapeutic alternatives in the bid to reduce the burden of infectious diseases associated with food borne bacteria.

Keywords: phytochemicals, antioxidants, plant spices, pork meats

Introduction

Pig meat (pork) is a popular meal eaten by many Nigerians and those in Owerri are not excluded. Pork is obtained from pig and it is known as "pig meat". It serves as food and is an important source of protein, vitamin and also fats for most people in many parts of the world (Yannick, Rawlings and Akwah, 2013). The percentage of fat in pork usually ranges from 10 – 16%, but can be much higher, depending on the level of trimming and other available factors. According to Murphy, Spungen, Bi and Barraj (2011), there are health benefits that can be derive from pork, they include muscle mass maintenance, and adequate intake of pork helps in the high - quality nutrient that may help preserve muscle mass and enhanced exercise performance. Vitamins like thiamin, selenium, vitamin B, niacin, phosphorus are found in pork as well as some other compounds like creatine, taurine, and cholesterol (Murphy, Spungen, Bi and Barraj, 2011). Many people in Nigeria especially in the South-Eastern part of the country like to consume pig meat that is why pig keeping and consumption are rapidly increasing, and pork joints are located on some busy streets and roads. Pork joints are a mix of pork butchering and a snack bar where ready- to - eat or take away food are sold. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans as medicinal ingredients and nutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage, and also contribute to the plant's colour, aroma and flavour.

There are chemicals that protect plants from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack, these chemicals are called as phytochemicals (Gibson, Wardel, and Watts, 1998; Mathai, 2000).Wide-ranging dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices (Mathai, 2000). Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, raspberries, beans and soy foods are also the common sources of phytochemicals (Moorachian, 2000). Phytochemicals accumulate in different parts of the plants, such as in the root, stem, leaf, flower, fruit and seed (Costa, Zia, Davin and Lewis, 1999). Examples of phytochemicals includes; flavonoids, saponins, alkaloids, tannins, steroids, glycosides, anthraquinone, terpenoids, to mention just a few.
Meanwhile, antioxidants are substances that when present at low concentrations, compared to those of the oxidizable substrate significantly delays or inhibits the oxidation of the substrate (Prior, Wu and Schaich, 2005). An important role of antioxidants is to suppress free radical−mediated oxidation by inhibiting the formation of free radicals by scavenging radicals. Radical scavenging action is dependent on both the reactivity and concentration of the antioxidant. Free radicals, particularly reactive oxygen species (ROS) have a greater impact on humans both from within the body and the environment. During metabolism, ROS such as superoxide (O^{2-}), hydroxyl (OH) and hydrogen peroxide (H_2O_2) can arise normally or sometimes the immune cells create them purposefully to neutralize the foreign bodies. Moreover, environmental factors such as pollution, radiation, cigarette smoke and herbicides can also generate free radicals. These ROS can damage essential proteins, DNA and lipids and cause various human diseases like Atherosclerosis (Moon and Shibamoto, 2009), cancer, liver injury, cardiovascular disease (Liao and Yin, 2000), neurodegenerative disorders and rheumatism as a result of 'oxidative stress'. Although, the body possesses defense mechanisms as enzymes and antioxidant nutrients, which arrest the damaging properties of ROS continuous exposure to chemicals and contaminants may increase the number of free radicals in the body beyond its ability to control and cause irreversible oxidative damages (Prior, Wu and Schaich, 2005).

Therefore, antioxidants with free radical scavenging activities may be relevant in the prevention and therapeutics of diseases where free radicals are implicated. WHO has recommended

the use of natural antioxidants that can delay or inhibit the lipids or other molecules oxidation by inhibiting the initiation or propagation of oxidative chain reactions (Liao and Yin, 2000).

This study reports on the effect of phytochemicals and antioxidants of indigenous spices *Capsicum frutescens* (Ghana Pepper), *Piper guineense* (*Uziza*), *Ocimum gratissimum* (Scent Leaf), *Allium cepa* (Onions)and *Gongronema latifolium* (*Utazi*) against food-borne bacterial isolates from pork meat sold in Owerri.

Materials and Methods

Collection of Plant Materials - Different plant materials (spices – *Capsicum frutescens*, *Piper guineense*, *Ocimum gratissimum*, *Allium cepa* and *Gongronema latifolium*) were purchased from the local market in Ihiagwa. Before sample collection, a preliminary survey was conducted with the spice dealers who had close contact with the farmers in the various regions to authenticate and ascertain the source of the spices. Spices were chosen based on their widespread use in local community (use as an additive with pork meat). The collected samples were disinfected, air dried in a shade and pulverized (blended) to increase the surface area. Each sample was weighed and soaked in different extraction solvents (99.5% ethanol, 99% methanol, and 99% dimethyl sulfoxide DMSO in the ratio 1:9 w/v. The mixtures were subjected to shaking at 25◦C for 48 h. For distilled water extracts, weighed samples were soaked in 80◦C distilled water for 30 min with intermittent shaking. Filtration was performed using Whatman filter paper (110 mm) and the filtrate subsequently centrifuged at 9500 rpm for 15 min at 4◦C. The supernatants (10% extracts) were obtained and stored at 10◦C until use.

Collection of Pork Meats - Samples of pork meat were purchased from different vendours from five locations in Owerri West (Obinze, Ihiagwa, Eziobodo, FUTO back gate and Umuchima). Five porkmeats each from three different areas in all the five location, that's 15 samples per location, making it a total of 75 samples (Kothari, 2014). It was purchased, placed in a sterile polythene bags and transported to the laboratory for immediate analysis.

Preparation of Media and Diluents - All media and diluents were prepared according to the manufacturer's specification (Atlas, 2010). Spread method was used as described by Prescott and Harley(2002).

Characterization of Test Organisms - Microbial isolates were characterized based on cultural (colonial), microscopic and biochemical methods with reference to standard manuals. The identities of the isolates were cross-matched with reference to standard manuals for the identification of bacteria (Cheesbrough, 2000; Buchanan and Gibbon, 2000; Harrigan and McCance, 2000).

Qualitative Phytochemical Screening of Spices

The method of Kumar et al.(2017) was adopted for the qualitative phytochemical screening of plant spices as given below in Table 3.1

Quantitative Phytochemical Screening of Spices

Watson and Sparkman (2007) method was adopted for the qualitative phytochemical screening of spices. 1g of the spice was weighed and transferred in a test tube and 25ml of ethanol was added. The test tube was allowed to react in a hotplate at 60^0C for 90mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. The extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution as dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000ul of pyridine of which 200ul was transferred to a vial for analysis.

The analysis of phytochemical was performed on a BUCK M910 Gas chromatography

equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% of the extracts diluted with acetonitrile was injected in a splitless mode. Relative quantity of the chemical compounds present in each of the extracts of was expressed as percentage based on peak area produced in the chromatogram.

Table 3.1: Qualitative Phytochemical Screening of Plant Spices

Parameters	Test	Observation	Inference
Alkaloids	1ml extract+ 2ml Meyers reagents + 1ml 1%HCL	Brick red	Alkaloid present
Flavonoid	3ml of extract + 1ml 10% NaOH	Yellow colouration	Flavonoid present
Tannin	1ml extract + 2 drops of 5% $FeCl_3$	Bluish-black colouration	Tannin present
Saponin	3ml extract + 5 drops of olive oil (emulsion test)	Formation of emulsion	Saponin present
Steroids	1ml extract+ 5 drops of conc. sulphuric acid (H_2SO_4)	Red colouration	Steroid present
Glycosides	5ml of 25% H_2SO_4 0.5ml extract + Fehling solution boil for 15mins	Brick red colouration	Glycoside present
Anthraquinone	2.5ml extract +5ml benzene + 2.5ml 5% NH_3 then agitate	Pink colouration	Anthraquinone present
Terpenoid	2ml extract + 2ml Salkowskis reagent	Brown colouration	Terpenoid present

Kumar et al.(2017)

Antioxidant Assay of Plant Extracts

The radical scavenging activity of the plant extracts was determined by using 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) assay according to Boonchum et al. (2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as reference.0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol. Different volumes (2 – 20µl) of plant extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The percentage (%) radical scavenging activity of the plant extracts was calculated using the following formula:

$$\%RSA = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100$$

Where, RSA is the Radical Scavenging Activity; *Abs control* is the absorbance of DPPH radical + ethanol; *Abs sample* is the absorbance of DPPH radical + plant extract.

Preparation of Test Organism - Test organisms for standardization from slant agar were sub-cultured onto a nutrient agar at 37°C for 24 h. Organisms were further grown on a slant for preservation (Gotep et al., 2009). Test isolates were standardized by McFarland method. McFarland solution consists of Barium Chloride and Sulphuric Acid. Mcfarland 0.5 which is equivalent to a cell density of 1.5×10^8 Cfu/ml was used.

Antibacterial Activity Testing Using the Agar Well Method - Srinivasan et al., 2001 method was adopted. The isolated bacteriafrom their selective media were inoculated into 10 ml of sterile nutrient broth and incubated at 37°C for 18 hours (0.5 Mc Far-land Standards). Using a sterile cotton swab, the nutrient broth culture was swabbed on the surface of sterile Mueller-Hinton Agar (MHA) plates. Agar wells were prepared with the help of a sterilized cork borer with a 6 mm diameter. Using a micropipette, 100 microlitres of different concentrations of nanoparticle plant spice extracts (500, 250, 125, 62.5 µg/ml) were added to different wells on the plate with Ciprofloxacin as control. The plates were incubated in an upright position at 37° C for 24 hours. The diameter of inhibition zones was measured in mm and the results were recorded.

Results and Discussion

Total Heterotrophic Bacterial Counts of Bacteria isolated from Pork Meat

Table 4.1 to Table 4.5 shows the total plate count of bacteria isolated from pork meat in Obinze, Ihiagwa, Eziobodo, FUTO backgate and Umuchima respectively. Table 4.1 shows the total plate count of bacteria isolated from pork meat in Obinze. The media used were Nutrient Agar (NA), *Salmonella Shigella* Agar (SSA), Eosine Methylene Blue Agar (EMBA), Thiosulphate Bile Salt Sucrose Agar (TCBS), Cetrimide Agar (CA) and Mannitol Salt Agar (MSA). From the table, the total count for NA ranges from 2.50×10^7 – 2.99×10^7 Cfu/g, the total count for SSA ranges from 3.6×10^3 – 6.9×10^3 Cfu/g, the total count for EMBA ranges from 1.26×10^4 – 1.92×10^4 Cfu/g, the total count for TCBS ranges from 2.50×10^4 – 2.98×10^4 Cfu/g, the total count for CA ranges from 2.90×10^3 – 2.20×10^4 Cfu/g while the total count for MSA ranges from 3.10×10^3 – 2.98×10^4 Cfu/g.

Table 4.2 shows the total plate count of bacteria isolated from pork meat in Ihiagwa. The media used were Nutrient Agar (NA), *Salmonella Shigella* Agar (SSA), Eosine Methylene Blue Agar (EMBA), Thiosulphate Bile Salt Sucrose Agar (TCBS), Cetrimide Agar (CA) and Mannitol Salt Agar (MSA). From the table, the total count for NA ranges from 2.40×10^7 – 2.99×10^7 Cfu/g, the total count for SSA ranges from 4.3×10^3 – 5.5×10^3 Cfu/g, the total count for EMBA ranges from 1.3×10^4 – 1.9×10^4 Cfu/g, the total count for TCBS ranges from 2.20×10^4 – 2.88×10^4 Cfu/g, the total count for CA ranges from 4.40×10^3 – 2.00×10^4 Cfu/g while the total count for MSA ranges from 2.50×10^3 – 2.80×10^4 Cfu/g.

Table 4.3 shows the total plate count of bacteria isolated from pork meat in Eziobodo. The media used were Nutrient Agar (NA), *Salmonella Shigella* Agar (SSA), Eosine Methylene Blue Agar (EMBA), Thiosulphate Bile Salt Sucrose Agar (TCBS), Cetrimide Agar (CA) and Mannitol Salt Agar (MSA). From the table, the total count for NA ranges from 2.45×10^7 – 2.92×10^7 Cfu/g, the total count for SSA ranges from 1.50×10^3 – 4.50×10^3 Cfu/g, the total count for EMBA ranges from 1.40×10^4 – 1.82×10^4 Cfu/g, the total count for TCBS ranges from 2.44×10^4 – 2.90×10^4 Cfu/g, the total count for CA ranges from 4.90×10^3 – 2.10×10^4 Cfu/g while the total count for MSA ranges from 1.20×10^3 – 2.90×10^4 Cfu/g.

Table 4.4 shows the total plate count of bacteria isolated from pork meat in Backgate. The media used were nutrient agar (NA), *Salmonella Shigella* agar (SSA), Eosine Methylene Blue Agar (EMBA), Thiosulphate Bile Salt Sucrose Agar (TCBS), Cetrimide Agar (CA) and Mannitol Salt

Agar (MSA). From the table, the total count for NA ranges from 2.30×10^7 – 2.99×10^7 Cfu/g, the total count for SSA ranges from 1.60×10^3 – 5.90×10^3 Cfu/g, the total count for EMBA ranges from 1.70×10^4 – 1.97×10^4 Cfu/g, the total count for TCBS ranges from 2.47×10^4 – 2.90×10^4 Cfu/g, the total count for CA ranges from 5.70×10^3 – 1.75×10^4 Cfu/g while the total count for MSA ranges from 1.80×10^3 – 4.90×10^3 Cfu/g.

Table 4.5 shows the total plate count of bacteria isolated from pork meat in Umuchima. The media used were Nutrient Agar (NA), *Salmonella Shigella* Agar (SSA), Eosine Methylene Blue Agar (EMBA), Thiosulphate Bile Salt Sucrose Agar (TCBS), Cetrimide Agar (CA) and Mannitol Salt Agar (MSA). From the table, the total count for NA ranges from 2.40×10^7 – 2.90×10^7 Cfu/g, the total count for SSA ranges from 1.70×10^3 – 6.50×10^3 Cfu/g, the total count for EMBA ranges from 1.60×10^4 – 1.92×10^4 Cfu/g, the total count for TCBS ranges from 2.40×10^4 – 2.75×10^4 Cfu/g, the total count for CA ranges from 2.80×10^3 – 1.90×10^4 Cfu/g while the total count for MSA ranges from 2.90×10^3 – 8.90×10^3 Cfu/g.

Figure 4.1: Some Culture Plates and Broth Culture

Figure 4.1 illustrates images of same culture plates and broth solutions. The number of occurrence of the isolates from pork meats are; *Salmonella* sp (102), *Escherichia coli* (277), *Shigella* sp (98), *Staphylococcus* sp (194), *Bacillus* sp (169), *Enterococcus* sp (76), *Pseudomonas* sp (114) and *Vibrio* sp (558).

In this study, some bacteria were implicated in the pork meat (Bae et al., 2022) sold by different vendors in various locations (obinze, Ihiagwa, Umuchima, Backgate and Eziobodo). This agrees with a study which presented that various foodborne bacteria, including *Salmonella sp*, *Clostridium perfringens*, and *Staphylococcus aureus*, were isolated from edible pig samples (Im, Seo, Bae and lee, 2016). The percentage occurrences of these bacteria are listed thus: *Salmonella* sp (6.42%), *Escherichia coli* (17.44%), *Shigella* sp (6.17%), *Staphylococcus* sp (7.05%), *Bacillus cereus* (4.72%), *Bacillus subtilis* (5.92%), *Enterococcus* sp (4.79%), *Staphylococcus aureus* (5.16%), *Vibrio cholerae* (18.14%), *Pseudomonas* sp (7.18%) and *Vibrio parahaemolyticus* (17.00%) (Ballash, Albers, Mollenkopf, Sechrist, Adams and Wittum, 2021). The percentage occurrences of bacteria implicated in pork meat in their decreasing order; *Vibrio cholerae* >*Escherichia coli*> *Vibrio parahaemolyticus* >*Pseudomonas* sp >*Staphylococcus* sp >*Salmonella* sp >*Shigella* sp >*Bacillus subtilis* >*Staphylococcus aureus* > *Bacillus cereus* (Safiya, 2011). The total heterotrophic bacteria count (lowest - highest) for

each location is stated as follows; Obinze (2.9×10^3 – 2.99×10^7Cfu/g), Ihiagwa (2.5×10^3 – 2.99×10^7Cfu/g), Eziobodo (1.2×10^3 – 2.92×10^7Cfu/g), Backgate (1.8×10^3 – 2.99×10^7Cfu/g)and Umuchima (2.9×10^3 – 2.9×10^7Cfu/g) (Zwirzitz et al., 2020; Self, Lunar, Fothergill Holt and Vieira, 2017).

Table 4.1: Total Count of Bacteria isolated from Pork Meat in Obinze

	Isolation Medium					
CODES	**NA**	**SSA**	**EMBA**	**TCBS**	**CA**	**MSA**
OA1	2.96×10^7	3.6×10^3	1.36×10^4	2.87×10^4	6.30×10^3	7.10×10^3
OA2	2.98×10^7	5.4×10^3	1.90×10^4	2.80×10^4	2.02×10^4	2.72×10^4
OA3	2.70×10^7	5.2×10^3	1.74×10^4	2.70×10^4	2.90×10^3	8.80×10^3
OA4	2.80×10^7	3.9×10^3	1.50×10^4	2.60×10^4	9.60×10^3	7.40×10^3
OA5	2.50×10^7	4.7×10^3	1.55×10^4	2.50×10^4	2.15×10^4	2.64×10^4
OB1	2.99×10^7	4.4×10^3	1.92×10^4	2.90×10^4	2.20×10^4	2.77×10^4
OB2	2.89×10^7	4.5×10^3	1.79×10^4	2.87×10^4	5.70×10^3	2.98×10^4
OB3	2.85×10^7	4.9×10^3	1.63×10^4	2.82×10^4	8.30×10^3	2.88×10^4
OB4	2.58×10^7	5.7×10^3	1.33×10^4	2.72×10^4	8.90×10^3	2.70×10^3
OB5	2.49×10^7	6.2×10^3	1.37×10^4	2.66×10^4	9.70×10^3	7.10×10^3
OC1	2.94×10^7	6.6×10^3	1.87×10^4	2.69×10^4	9.20×10^3	7.50×10^3
OC2	2.91×10^7	6.7×10^3	1.92×10^4	2.65×10^4	5.60×10^3	2.75×10^4
OC3	2.79×10^7	6.9×10^3	1.85×10^4	2.79×10^4	6.50×10^3	2.89×10^4
OC4	2.77×10^7	4.7×10^3	1.26×10^4	2.85×10^4	7.10×10^3	3.10×10^3
OC5	2.78×10^7	5.2×10^3	1.37×10^4	2.98×10^4	7.70×10^3	3.50×10^3

Key: NA = Nutrient Agar, SSA = *Salmonella Shigella*, CA= Cetrimide Agar, MSA = Mannitol Salt Agar, EMBA = Eosine Methylene Blue Agar, TCBS= Thiosulfate Citrate Bilatesalts Sucrose Agar, 0A1 = Obinze A1

Table 4.2: Total Count of Bacteria isolated from Pork Meat in Ihiagwa

	Isolation Medium					
CODES	NA	SSA	EMBA	TCBS	CA	MSA
IA1	2.9×10^7	4.4×10^3	1.8×10^4	2.8×10^4	6.8×10^3	2.6×10^4
IA2	2.8×10^7	5.5×10^3	1.79×10^4	2.6×10^4	1.9×10^4	2.5×10^3
IA3	2.77×10^7	4.9×10^3	1.7×10^4	2.4×10^4	5.9×10^3	2.6×10^4
IA4	2.7×10^7	4.2×10^3	1.9×10^4	2.3×10^4	2.0×10^4	2.7×10^4
IA5	2.65×10^7	5.0×10^3	1.82×10^4	2.2×10^4	5.7×10^3	2.9×10^3
IB1	2.91×10^7	5.1×10^3	1.88×10^4	2.5×10^4	5.5×10^3	2.8×10^3
IB2	2.88×10^7	4.7×10^3	1.62×10^4	2.47×10^4	1.1×10^4	3.5×10^3
IB3	2.52×10^7	4.4×10^3	1.5×10^4	2.6×10^4	4.4×10^3	2.7×10^3
IB4	2.6×10^7	4.8×10^3	1.49×10^4	2.7×10^4	5.4×10^3	2.4×10^4
IB5	2.71×10^7	4.3×10^3	1.3×10^4	2.88×10^4	4.9×10^3	2.5×10^4
IC1	2.99×10^7	4.8×10^3	1.4×10^4	2.5×10^4	5.2×10^3	2.7×10^3
IC2	2.91×10^7	4.9×10^3	1.5×10^4	2.3×10^4	1.12×10^4	2.8×10^3
IC3	2.4×10^7	5.0×10^3	1.6×10^4	2.5×10^4	4.7×10^3	3.1×10^3
IC4	2.6×10^7	5.2×10^3	1.7×10^4	2.7×10^4	4.4×10^3	3.9×10^3
IC5	2.68×10^7	4.8×10^3	1.82×10^4	2.7×10^4	4.5×10^3	2.8×10^4

Key: NA = Nutrient Agar, SSA = *Salmonella Shigella*, CA= Cetrimide Agar, MSA = Mannitol Salt Agar, EMBA = Eosine Methylene Blue Agar, TCBS= Thiosulfate Citrate Bilatesalts Sucrose Agar, IA1 = Ihiagwa A1

Table 4.3: Total Count of Bacteria isolated from Pork Meat in Eziobodo

	Isolation Medium					
CODES	NA	SSA	EMBA	TCBS	CA	MSA
EA1	2.88×10^{7}	3.1×10^{3}	1.8×10^{4}	2.9×10^{4}	7.3×10^{3}	2.7×10^{4}
EA2	2.7×10^{7}	1.7×10^{3}	1.82×10^{4}	2.6×10^{4}	2.0×10^{4}	1.5×10^{3}
EA3	2.9×10^{7}	1.6×10^{3}	1.87×10^{4}	2.7×10^{4}	2.1×10^{4}	2.6×10^{4}
EA4	2.7×10^{7}	1.7×10^{3}	1.77×10^{4}	2.8×10^{4}	8.9×10^{3}	2.5×10^{4}
EA5	2.9×10^{7}	1.5×10^{3}	1.8×10^{4}	2.9×10^{4}	8.6×10^{3}	2.3×10^{4}
EB1	2.69×10^{7}	3.5×10^{3}	1.72×10^{4}	2.88×10^{4}	9.2×10^{3}	1.2×10^{3}
EB2	2.9×10^{7}	3.9×10^{3}	1.6×10^{4}	2.7×10^{4}	1.0×10^{4}	1.45×10^{3}
EB3	2.92×10^{7}	4.5×10^{3}	1.65×10^{4}	2.6×10^{4}	1.2×10^{4}	1.6×10^{3}
EB4	2.45×10^{7}	3.8×10^{3}	1.69×10^{4}	2.69×10^{4}	1.3×10^{4}	2.9×10^{4}
EB5	2.69×10^{7}	4.2×10^{3}	1.72×10^{4}	2.8×10^{4}	1.5×10^{4}	2.74×10^{4}
EC1	2.66×10^{7}	4.5×10^{3}	1.68×10^{4}	2.9×10^{4}	2.0×10^{4}	2.6×10^{4}
EC2	2.52×10^{7}	3.8×10^{3}	1.59×10^{4}	2.59×10^{4}	4.9×10^{3}	2.45×10^{4}
EC3	2.49×10^{7}	3.2×10^{3}	1.52×10^{4}	2.44×10^{4}	5.2×10^{3}	2.7×10^{4}
EC4	2.92×10^{7}	2.7×10^{3}	1.48×10^{4}	2.59×10^{4}	6.5×10^{3}	1.6×10^{3}
EC5	2.89×10^{7}	2.9×10^{3}	1.4×10^{4}	2.6×10^{4}	7.5×10^{3}	1.7×10^{3}

Key: NA = Nutrient Agar, SSA = *Salmonella Shigella*, CA= Cetrimide Agar, MSA = Mannitol Salt Agar, EMBA = Eosine Methylene Blue Agar, TCBS= Thiosulfate Citrate Bilatesalts Sucrose Agar, EA1 = Eziobodo A1

Table 4.4: Total Count of Bacteria isolated from Pork Meat in Back Gate

	Isolation Medium					
CODES	NA	SSA	EMBA	TCBS	CA	MSA
BA1	2.96×10^{7}	1.6×10^{3}	1.7×10^{4}	2.65×10^{4}	6.2×10^{3}	3.7×10^{3}
BA2	2.99×10^{7}	4.4×10^{3}	1.84×10^{4}	2.72×10^{4}	1.44×10^{4}	1.8×10^{3}
BA3	2.5×10^{7}	3.0×10^{3}	1.9×10^{4}	2.5×10^{4}	8.9×10^{3}	4.4×10^{3}
BA4	2.4×10^{7}	3.5×10^{3}	1.97×10^{4}	2.47×10^{4}	1.75×10^{4}	4.2×10^{3}
BA5	2.3×10^{7}	4.8×10^{3}	1.85×10^{4}	2.79×10^{4}	7.2×10^{3}	4.5×10^{3}
BB1	2.9×10^{7}	4.0×10^{3}	1.75×10^{4}	2.77×10^{4}	5.7×10^{3}	4.9×10^{3}
BB2	2.89×10^{7}	1.9×10^{3}	1.71×10^{4}	2.78×10^{4}	1.57×10^{4}	2.7×10^{3}
BB3	2.97×10^{7}	2.7×10^{3}	1.82×10^{4}	2.85×10^{4}	1.14×10^{4}	3.5×10^{3}
BB4	2.5×10^{7}	3.2×10^{3}	1.83×10^{4}	2.8×10^{4}	8.8×10^{3}	3.1×10^{3}
BB5	2.43×10^{7}	3.3×10^{3}	1.85×10^{4}	2.69×10^{4}	7.5×10^{3}	3.9×10^{3}
BC1	2.44×10^{7}	3.9×10^{3}	1.98×10^{4}	2.71×10^{4}	1.11×10^{4}	3.7×10^{3}
BC2	2.54×10^{7}	4.5×10^{3}	1.87×10^{4}	2.8×10^{4}	1.25×10^{4}	3.2×10^{3}
BC3	2.7×10^{7}	5.9×10^{3}	1.88×10^{4}	2.9×10^{4}	1.39×10^{4}	3.5×10^{3}
BC4	2.44×10^{7}	5.7×10^{3}	1.79×10^{4}	2.6×10^{4}	1.57×10^{4}	3.8×10^{3}
BC5	2.55×10^{7}	5.5×10^{3}	1.68×10^{4}	2.8×10^{4}	8.7×10^{3}	4.7×10^{3}

Key: NA = Nutrient Agar, SSA = *Salmonella Shigella*, CA= Cetrimide Agar, MSA = Mannitol Salt Agar, EMBA = Eosine Methylene Blue Agar, TCBS= Thiosulfate Citrate Bilatesalts Sucrose Agar, BA1 = BackGate A1

Table 4.5: Total Count of Bacteria isolated from Pork Meat in Umuchima

	Isolation Medium					
CODES	NA	SSA	EMBA	TCBS	CA	MSA
UA1	2.7×10^{7}	1.7×10^{3}	1.9×10^{4}	2.5×10^{4}	6.5×10^{3}	2.9×10^{3}
UA2	2.8×10^{7}	5.7×10^{3}	1.87×10^{4}	2.49×10^{4}	7.2×10^{3}	8.7×10^{3}
UA3	2.7×10^{7}	4.9×10^{3}	1.8×10^{4}	2.6×10^{4}	1.5×10^{4}	4.5×10^{3}
UA4	2.6×10^{7}	4.5×10^{3}	1.7×10^{4}	2.7×10^{4}	1.9×10^{4}	3.9×10^{3}
UA5	2.65×10^{7}	2.9×10^{3}	1.6×10^{4}	2.45×10^{4}	2.9×10^{3}	4.2×10^{3}
UB1	2.75×10^{7}	3.3×10^{3}	1.9×10^{4}	2.4×10^{4}	3.5×10^{3}	5.2×10^{3}
UB2	2.8×10^{7}	3.5×10^{3}	1.92×10^{4}	2.6×10^{4}	4.9×10^{3}	2.9×10^{3}
UB3	2.49×10^{7}	4.0×10^{3}	1.8×10^{4}	2.5×10^{4}	1.4×10^{4}	8.7×10^{3}
UB4	2.5×10^{7}	5.4×10^{3}	1.5×10^{4}	2.47×10^{4}	1.0×10^{4}	8.9×10^{3}
UB5	2.6×10^{7}	6.2×10^{3}	1.7×10^{4}	2.56×10^{4}	8.9×10^{3}	4.9×10^{3}
UC1	2.9×10^{7}	6.5×10^{3}	1.9×10^{4}	2.65×10^{4}	9.8×10^{3}	5.5×10^{3}
UC2	2.7×10^{7}	6.2×10^{3}	1.8×10^{4}	2.72×10^{4}	4.5×10^{3}	5.7×10^{3}
UC3	2.6×10^{7}	1.9×10^{3}	1.7×10^{4}	2.75×10^{4}	5.4×10^{3}	8.0×10^{3}
UC4	2.5×10^{7}	2.0×10^{3}	1.6×10^{4}	2.73×10^{4}	2.8×10^{3}	4.5×10^{3}
UC5	2.4×10^{7}	2.5×10^{3}	1.9×10^{4}	2.65×10^{4}	3.5×10^{3}	4.2×10^{3}

Key: NA = Nutrient Agar, SSA = *Salmonella Shigella*, CA= Cetrimide Agar, MSA = Mannitol Salt Agar, EMBA = Eosine Methylene Blue Agar, TCBS= Thiosulfate Citrate Bilatesalts Sucrose Agar, UA1 = Umuchima A1

Phytochemicals and Antioxidant Assay of Plant Samples - Table 4.9 shows the qualitative phytochemicals results of plant samples. The plant samples were test for eight phytochemicals which include; alkaloids, flavonoids, tannins, steroids, glycosides, anthraquinones, saponins and terpenoids. From the table, alkaloids are absent in utazi, flavonoids are absent in uziza, ghana pepper and onions, tannins are absent in scent leaf, steroids are absent in scent leaf and utazi, glycosides are present in ghana pepper, anthraquinones are absent in scent leaf, utazi and onions while saponins and terpenoids are present in all the plant samples.

Figure 4.3 shows the quantitative phytochemical analysis of the plant samples. The value range for the different phytochemicals are as follows; Saponins (3.29 – 7.26), Alkaloids (1.34 – 6.11), Tannins (5.20 – 6.45), Flavonoids (0.18 – 6.16), Terpenoids (3.47 – 6.13), Anthraquinone (0.13 - 7.17), Glycoside (1.26 – 8.42) and Steroids (0.47 – 6.52). Phytochemicals are biologically active organic substances found in plants used by humans as food, which may be beneficial for health, but for which no specific human deficiency disorder has been identified. Phytochemicals are recognized as bioactive components in plant spices which can be used as traditional herbal medicines (Kaefer and Milner, 2011).All plant samples contains phytochemicals and exhibited antioxidant properties. The importance of bioactive compounds like alkaloids, phenolics, flavonoids and some other secondary metabolites have been reported by Khare and Dalziel (2007). The presence of these phytochemicals is an added advantage to these plant spices in food. These phytochemicals have been reported to exert strong antibacterial activity against several microbes associated with oral diseases (Lobo et al., 2010). Results of phytochemical study showed that saponin and terpenoids were present in all plant samples (uziza, scent leaf, ghana pepper, utazi, onions) (Ababutain,2011). Alkaloids was present only in uziza, ghana pepper and onions, flavonoids were present only in scent leaf and utazi, tannin was present in all the plant samples except scent leaf, steroids was present in uziza, ghana pepper and onions, glycosides was present in only utazi while

anthraquinine was present in uziza and ghana pepper (Liliwirianis, Zain, Kassim and Karim, 2011; Tajkarimi, Ibrahim & Cliver, 2010). In a decreasing order, the phytochemicals are as follows; Saponins > Terpenoids > Tannins > Flavonoids > Steroids > Alkaloids > Glycosides > Anthraquinone (Besong et al., 2016). Onions has the highest level of phytochemicals while scent leaf possesses the least phytochemical property (Adeyemi, 2011). The concentration of these phytochemicals ranges from (0.13±0.01) – (8.71±0.01).

Figure 4.4 shows the Radical Scavenging Activities of the different plant spices. The range of the RSA % values are as follows; Onions (66.85 – 83.80), Utazi (48.47 – 62.03), Uziza (1.07 – 31.84), Ghana Pepper (67.80 – 73.22) and Scent Leaf (63.10 – 65.42). All plant spices in this study exerted antioxidant properties (Sigh, 2007). In a decreasing order in relation to their antioxidant properties, the plant spices are as follows; Onions (83.80%±0.04) > Ghana Pepper (73.22%±0.03) > Scent Leaf (65.42%±0.02) > Utazi (62.03%±0.02) > Uziza (31.84%±0.01) (Srinivasan, 2014; Pal & Verma, 2013). Antioxidants have the ability to scavenge free radicals in the human body and have been suggested to contribute to the protective effect of plant-based foods on diseases (Okigbo and Igwe, 2007).This report also demonstrated antimicrobial activities of the plant spices on the bacteria from the pork meat. These results were affirmed by the phytochemicals and antioxidants inherent in the spices (Anyawu and Nwosu, 2014).This implies that these plant spices possess the ability to exert antimicrobial properties (Rao, 2003).

Table 4.9: Qualitative Phytochemicals Results of Plant Samples

SAMPLES	UZIZA	SCENT LEAF	GHANA PEPPER	UTAZI	ONIONS
ALKALOIDS	+	-	+	-	+++
FLAVONOIDS	-	++	-	+++	-
TANNINS	++	-	++	++	+++
SAPONINS	+++	+++	+++	+++	++
STEROIDS	+	-	+	-	+++
GLYCOSIDES	-	-	-	++	-
ANTHRAQUINONE	+	-	+	-	-
TERPENOIDS	++	+	+++	+	+

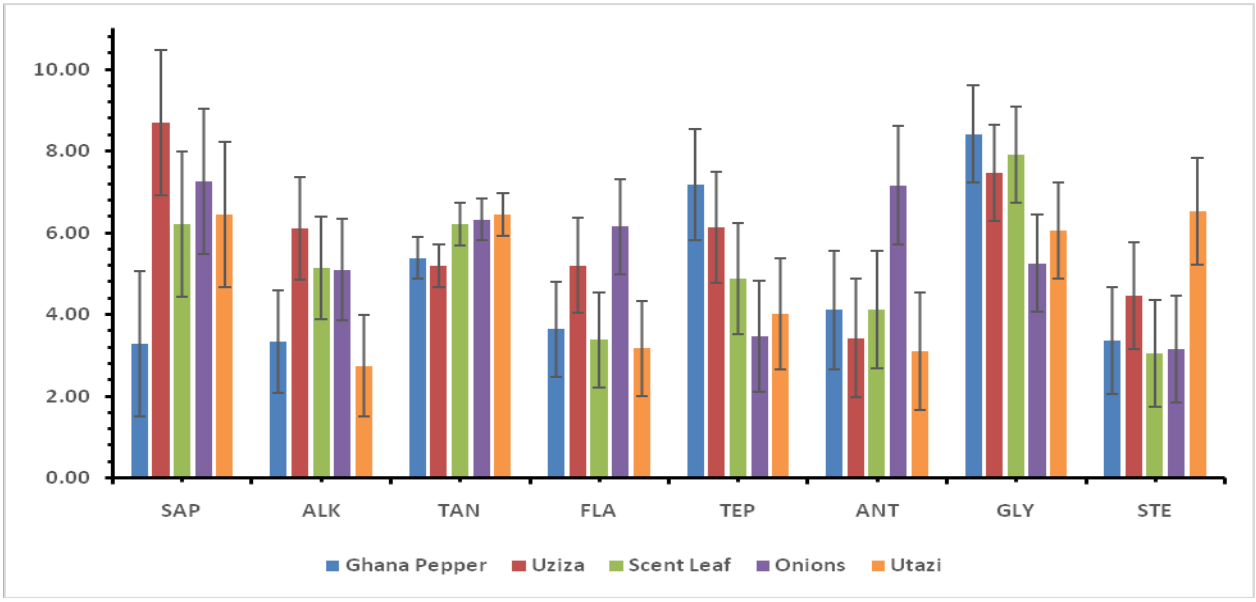

Figure 4.3: Quantitative Phytochemical Analysis of Samples (n=2)
Key: SAP, Saponin; AKL, Alkaloid; TAN, Tannin; FLA, Flavonoid; TEP, Terpenoid; ANT, Anthraquinone; GLY, Glycosides; STE, Steroid

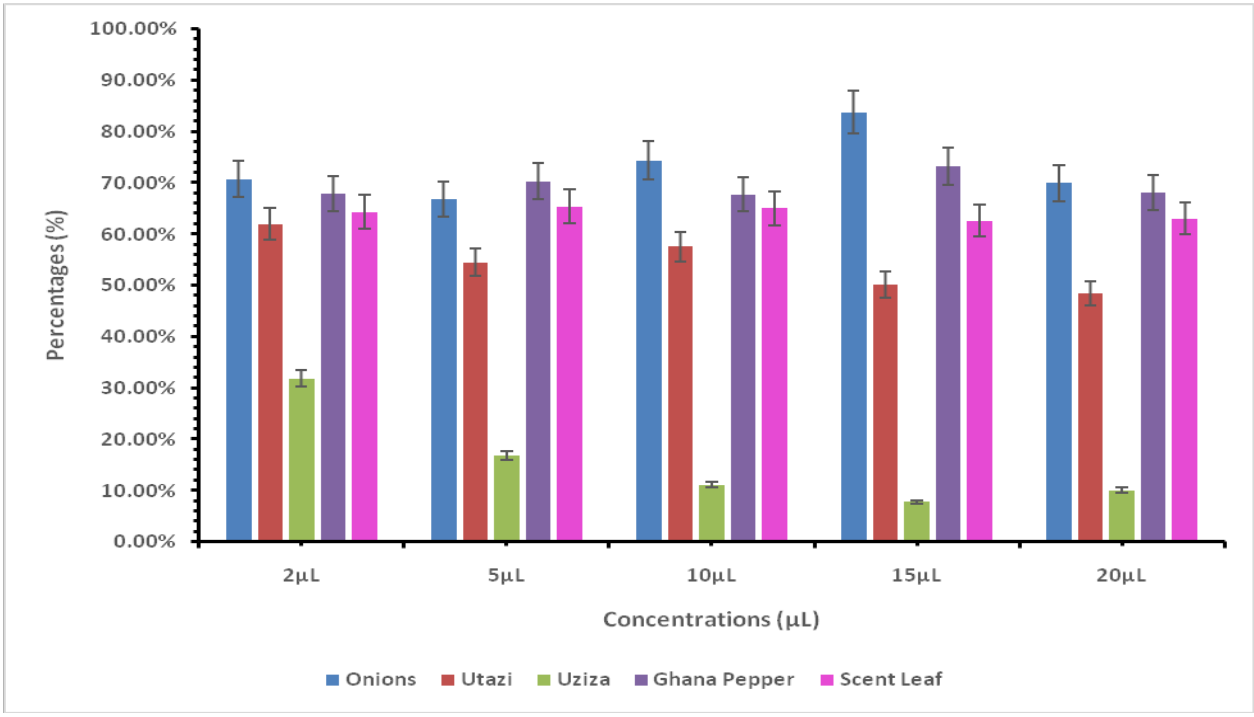

Figure 4.4: Radical Scavenging Activities of the Plant Spices (n=2)

Figure 4.8 shows the Zones of Inhibition of the spices against *Vibrio cholerae,* Figure 4.9 shows the Zones of Inhibition of the spices against *Salmonella* sp, Figure 4.10 shows the Zones of Inhibition of the spices against *Staphylococcus* sp, Figure 4.11 shows the Zones of inhibition of the spices against *E. coli*, Figure 4.12 shows the Zones of inhibition of the spices against *Vibrio cholerae*, Figure 4.13 shows the Zones of inhibition of the spices against *Salmonella* sp, Figure 4.14 shows the Zones of inhibition of the spices against *Staphylococcus* sp, Figure 4.15 shows the Zones of inhibition ofthe spices against *Vibrio parahaemolyticus* and Figure 4.16 shows the Zones of inhibition of the spices against *Pseudomonas* sp. In all figures, Ciprofloxacin (control) gave the highest zones of inhibition. This shows that all the spices exerts antibacterial effects against the bacterial isolates.

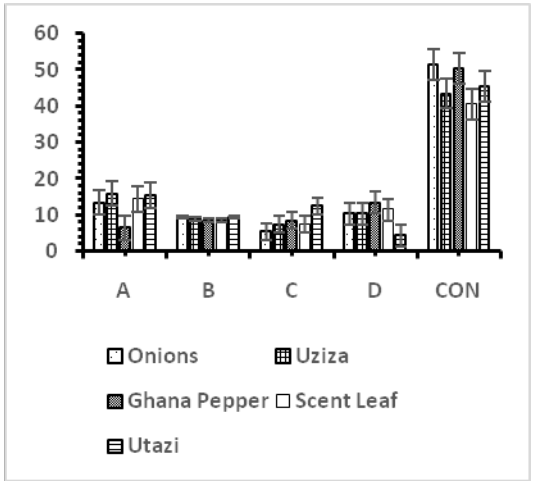

Fig.4.8: ZOI of Spices against *V. cholerae*

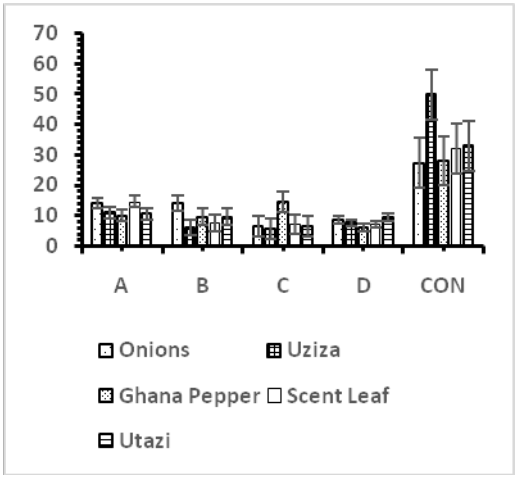

Fig. 4.9: ZOI of Spices against *Salmonella* sp

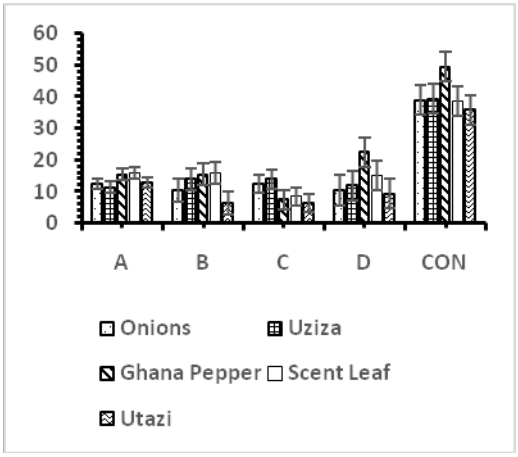

Fig. 4.10: ZOI of Spices against *Staphylococcus* sp

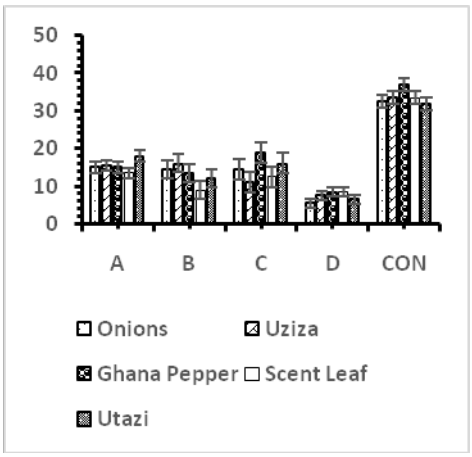

Fig. 4.11: ZOI of spices against *E. coli*

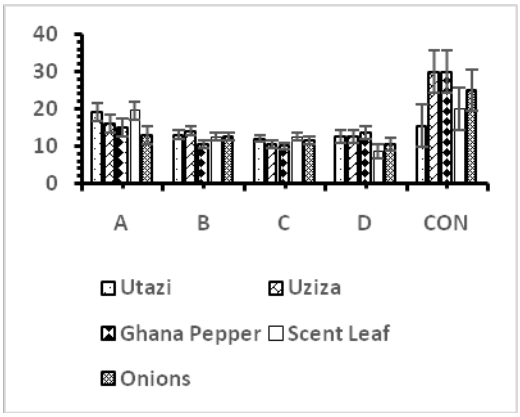

Fig. 4.12: ZOI of Spices against *V. Cholera*

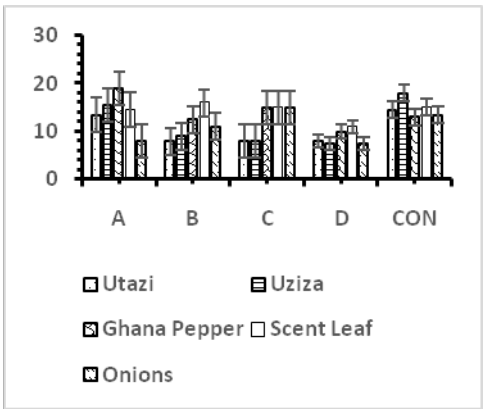

Fig. 4.13: ZOI of Spices against *Salmonella* sp

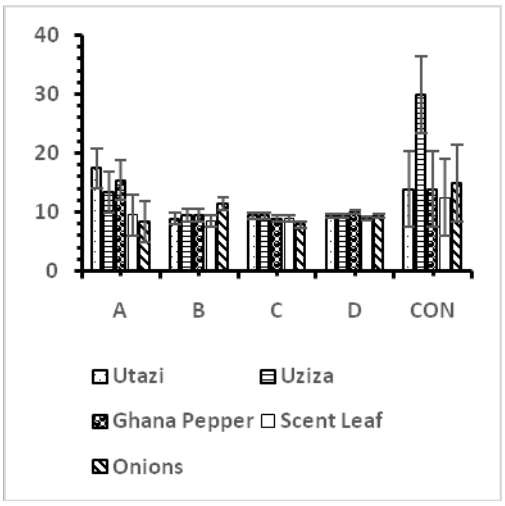

Fig. 4.14: ZOI of spices against *Staphylococcus* sp

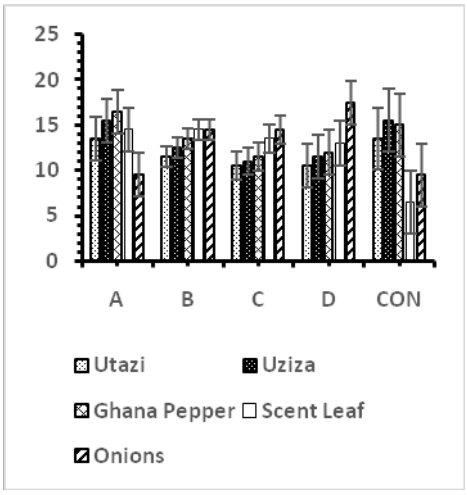

Fig 4.15: ZOI of Spices against *V. parahaemolyticus*

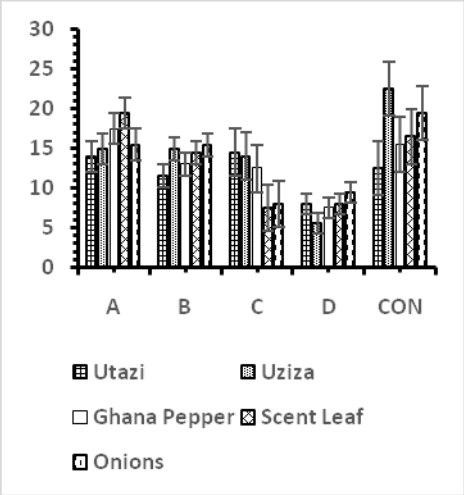

Fig. 4.16: ZOI of Spices against *Pseudomonas* sp

Conclusion

This study reveals that all spices contains phytochemicals and antioxidants indicating antibacterial activities. Their antibacterial activities were apparent from the zones of inhibition at different concentrations. Pork meats are sold in almost every joint in Owerri West Local Government Area (Imo State) and other parts of Nigeria. From this study, it is shown that the pork meats sold in the five locations (Ihiagwa, Obinze, Backgate, Umchima and Eziobodo) contains bacteria. This is a major concern to consumers as it has to do with their health (Clarence, Obinna and Shalom, 2005). Hygienic measures should be taken to make sure that pork meats are prepared in a bacteria-free condition and environment (Avraam, Lambrou, Jihang and Siddiqui, 2021). From this study, it is shown that indigenous plant spices exert antimicrobial and phytochemical properties that can either inhibit bacteria growth or annihilate bacteria. So, eating pork meats with some indigenous plant spices can slow down or reduce the effects of bacteria.

Also, the mesh used by most vendors in roasting pork meats should be put into consideration. This mesh should be kept indoors and heated properly before use. In practice, most vendors leave the mesh in an open place where they sell. Another alternative to eating pork meat is to buy it yourself and prepare by boiling it and adding any plant spice of your choice.

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