



Bacteriological and Antibiotic Resistance Evaluation of Bacteria Isolated from Fresh Meat Sold at Afor Oba Market, Anambra State

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ABSTRACT

This study was on bacteriological and antibiotic resistant evaluation of fresh meat sold at Afor Oba market, Anambra state. Four (4) samples of fresh meat were purchased for local sellers in Afor Oba Market and transferred to Tansian University Microbiology Laboratory for microbiological analysis. The samples were analyzed using Nutrient and MacConkey agar for bacteria isolates. The total viable count ranged between 2.7×10^5 to 4.5×10^5 on Nutrient Agar, while in MacConkey the total viable count ranged between 2.5×10^5 to 3.7×10^5 . The following bacteria were isolated: *Escherichia coli*, *Staphylococcus* spp, and *Salmonella* spp. Antibiotic sensitivity test was conducted and the following antibiotics names were recorded sensitive to the isolate *E. coli* with Chloraphenical having 14mm, Ciprofloxacin 18mm, Gentamycin having 21mm, Agabaxin 22mm, Zolacin 22mm, Tetracyclin 2mm, and was resistant to Amoxicillin and Septrin. The antibiotic names were recorded sensitive to the isolate *Staphylococcus* with Gentamycin having 3mm, Nitroforatoin 9mm, Cotrimacol 6mm, Ofcacin 6mm, Oerfuroxine 1mm, Cephalixin 4mm, tetracycline 2mm. The presence of these pathogenic bacteria is thus an indication of microbial contamination to the health of man. The presence of this bacteria isolate could be as a result of poor hygienic practices, unsterilized container used in storage of the meat, unhygienic slaughtering ground, poor personal hygiene of the vendors. Therefore, it is recommended that good hygiene practices is advocated for meat preparation/slaughtering to ensure human safety.

Keywords: Bacteriology, Meat, *Escherichia coli*, *Staphylococcus* spp, *Salmonella* spp.

INTRODUCTION

The fresh meat is often referred to as the meat that is obtained from the flesh of mammals and has more protein myoglobin content than white meat. The various types of fresh meat include beef, pork, mutton, veal, lamb, and goat. According to the National Chicken Council of the U.S, the per capita consumption of fresh meat in the year 2018 was 109.5 pounds. The U.S. was the largest consumer and producer of beef worldwide in the year 2018. The increasing demand of consumers towards convenience food has positively impacted the consumption rate of fresh meat (Abouzeed, *et al.*, 2002).

Fresh meat is the choice of meat over the other white flesh, which is procured from the fowl and duck. The comparatively higher protein content in fresh meat has nudged the consumers to shift towards beef and other red meats, thereby increasing its consumption. The highest protein content is found in pork with 29.41gms of protein per 100gms of meat consumed. The fresh meat is sold under the Halal and Kosher label-claims, in the middle eastern regions - leading to its market expansion (Adzitey, *et al.*, 2011). Microorganisms that cause deterioration have been identified as bacteria and fungi. In addition, various bacteria of public health concern such as *Salmonella* sp., *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, pathogenic strains of *Escherichia coli* and enterotoxigenic strains of *Staphylococcus aureus* may also be found in improperly stored food products (Agu *et al.*, 2014; Anaukwu *et al.*, 2015; Awah *et al.*, 2016; Agu *et al.*, 2022).

With the rising cost of meat and meat products and the prevailing population pressure in Nigeria as in other less developed countries has resulted in an increasing demand for wild under-exploited nutritious plant products with aesthetic and organoleptic appeal in daily diet (Enujiugha, 2005; Okpalla *et al.*, 2012; Agu *et al.*, 2013; Mbachu *et al.*, 2014; Anaukwu *et al.*, 2015). Fresh meat forms part of the habitual balanced diet for many adults living in the UK and Ireland. It is recognised that over many years of evolution, humans have adapted to consuming large quantities of lean fresh meat (Ateba, *et al.*, 2011). Recently, a number of epidemiological studies have associated red and processed meat consumption with the development of two of the major chronic diseases in the Western world; CVD and colon cancer (Banerjee, *et al.*, 2001). Constituents of fresh meat that have been proposed to be responsible for these associations include the fat content, fatty acid composition and the possible formation of carcinogenic compounds, such as heterocyclic amines (HCAs), by cooking meat at high temperatures (Beach, *et al.*, 2002). Although there are many studies documenting these associations, results are not always consistent and there are several methodological issues which could limit their findings. In the same way as the risks to health of fresh meat consumption must be evaluated, there are many health benefits which are equally as important in establishing public health messages in relation to fresh meat consumption (Bhandare, *et al.*, 2007).

The consumers changing preferences towards fresh meat owing to its better taste and mouth feel are expected to drive its market. The increasing disposable income of the consumers in developing economies has allowed them to spend more on animal-based products, consequently favouring the sales of red meat. According to the International Agency for Research on Cancer (IARC) in 2015, the ingestion of fresh meat could cause colorectal cancer. The carcinogenic and cardiovascular risks associated with the consumption of fresh meat is a major restraint for the global fresh meat market (Biswas, *et al.*, 2011).

Aim and Objectives of the Study

Aim

Bacteriological and antibiotic resistant evaluation of bacteria isolated from fresh meat sold at Afor Oba Market, Anambra State

Objectives of the Study

1. To isolate bacteria associated with fresh meat sold in Afor Oba Market, Anambra State.
2. To characterize the bacteria isolates
3. To evaluate the antibiotic resistant of the bacterial isolates

MATERIALS AND METHOD

Study Area

The study area is Afor Oba Market, Anambra state in the South East geopolitical zone of Nigeria.

Sources of Samples

The samples were purchased from four (4) different vendors from Afor Oba Market. This site was chosen because they are highly patronized by consumers.

Sample Collection

Four (4) fresh meat samples were purchased from different vendors mentioned above and were transported immediately to Tansian University Laboratory for bacteriological and antibiotic analysis.

Sterilization of Materials

The laboratory apparatus necessary for analysis were sterilized with autoclave at temperature 121°C for 15 minutes. This was done to remove and kill any microorganism that might have contaminated the laboratory apparatus. The entire working surface including the work bench was equally disinfected with 70% ethanol.

Analysis Procedure

Media Preparation: The different media which includes Nutrient agar and MacConkey agar was used in culturing bacteria. With the guide of the manufacturing instructions, 5.0g of Nutrient agar was added into 100ml of distilled water and was autoclave at 121°C for 15 minutes and allow cooling.

Microbial Analysis

Analysis of Sample: Each sample was serially diluted using sterile distilled water diluents. Nine milliliter (9ml) of distilled water was measured out into each test tube labeled 10-1, 10-2, and 10-3 Using separate sterile pipettes; 1ml of the meat sample was measured out into the first test tubes and properly mixed. Using a different pipette, 1ml from the first test tube was pipette into the second test tube already containing 9ml of distilled water. Using a different pipette, 1ml from the second test tube was pipette into the third test tube and lastly 1ml from the third test tube was pipette and then discards.

This was followed by pour plate technique.

Isolation of Contaminating Micro Organisms

Exactly (0.1ml) of the inoculum (10^{-5}) was poured into Nutrient Agar and spread evenly on it with the aid of a sterile glass spreader. The plate was allowed to dry for 15minutes. The plates were incubated at 35°C for 24 hours (1 day). The same were done for MacConkey. An un-inoculated agar plate of both Nutrient and MacConkey agar served as control.

After the incubation period, the plates were brought out of the incubators and the colonies were counted using a colony counter device and each count was expressed in colony forming unit per ml (cfu/ml).

Pure cultures were obtained by sub-culturing the different colonies into nutrient agar plate via streaking method. The plates were incubated at 35°C for 24 hours (1 day).

Sub-Culturing of the Cultures

Purification of Isolates: After incubation, colonies developing on plates were randomly picked and sub-cultured on fresh nutrient agar to obtain pure culture of the isolates. Using wire loop, it was inoculated on Petri dishes containing Nutrient agar for coliform/enterobacteriaceae counts at 37°C for 24 hours. After purification the isolates were maintained using nutrient agar slant and were kept at 4°C for identification.

Identification of Various Isolates

Identification of isolates was based on cultural features (such as motility, Gram-reaction, cell arrangement and shape) and biochemical features. The results obtained were then compared with standard references for proper identification of the isolates; Bergey's manual was used for bacteria identification.

The following biochemical tests were carried out for the characterization and identification of the organisms according to the method of (Cheesbrough, 2016).

- I. Catalase test
- II. Oxidase test
- III. Indole test
- IV. Sugar fermentation
- V. Coagulase test
- VI. Gram's staining

Catalase Test:

This test was useful in differentiating bacteria that produce the enzymes catalases such as *staphylococcus* species from non-catalases producing bacteria such as *streptococcus* species. Catalase acts as a catalyst in the breakdown of hydrogen peroxide and water according to (Cheesbrough, 2006).

Procedure:

- ✓ Two millimeters of hydrogen peroxide solution was poured in a test tube.
- ✓ With the aid of a sterile glass rod/wire loop, several colonies of the test organisms was collected from the nutrient agar plate and immersed in the hydrogen peroxide
- ✓ Bubbling was checked
- ✓ Active bubbling of the mixture indicates positive results.

Oxidase Test

This test was always used in the identification of bacteria that produce the enzyme cytochrome oxidase. To perform this test, a piece of filter paper was soaked with a few drops of oxidase reagents (1% tetramethyl p-phenylenediamine dihydrochloride) according to (Cheesbrough, 2006).

A colony of the test organism was then smeared on the filter paper, when the organism is oxidize producing the phenylenediamine, the reagents will be oxidized to deep purple colour.

Procedure:

- ✓ A piece of filter paper was placed in a clean petri dish and two drops of freshly prepared oxidize reagent was placed on it.
- ✓ Development of blue purple colour was checked for within few seconds.
- ✓ The blue purple colour produced indicates a positive result.

Indole Test:

This test is important in the identification of entero bacteria especially, *Escherichia coli*, that are able to breakdown the amino acid, tryptophan according to (Cheesbrough, 2006).

In this test, the test organisms cultured in a medium, which contains tryptophan, indole production is detected by Kovac's or Ehrlich's reagent containing 4(p) dimethyl aminobenzaldehyde, this reacts with the indole to produce a red coloured compound.

Procedure:

1. The test organism was inoculated into a bijou bottle containing 3ml of sterile Tryptone water.
2. This was incubated at 37°C for 48 hours

Citrate Test:

Simmon's culture medium, a modification of koser's medium with agar was used, agar was added to solidify the medium that was dispersed in test tubes, autoclaved at 121°C for 15 minutes and allowed to set as slopes. The Simon's medium constituted loser's medium (11g), agar (30g) and bromothymol blue (40ml) as an indicator according (2006).

The solidified medium was inoculated with the test organism and incubated at 37°C for 96 hours. Blue colour and streak of growth indicated Positive result.

Gram Staining:

Gram staining is a useful tool for identification of bacteria in specimen and cultures by their gram reaction and morphology. This technique divides bacteria into two groups- Gram positive and Gram negative.

Gram Positive and Gram Negative: the classification is based on the differences in the composition and permeability of bacteria cell walls. The Gram positive bacteria retain the stain, crystal violet while the Gram negative bacteria are discolored and are able to take up the counter stain according to (Cheesbrough, 2006).

Procedure:

- ❖ A thin smear of the culture was made on a clean grease free slide and the film was allowed to air dry and fixed by gently passing the slide over the flame three times.
- ❖ The smear was covered with crystal violet solution and allowed to stain for 60 seconds.
- ❖ Excess stain was dipped off and washed with clean water.
- ❖ The smear was stained with lugol's iodine and allow for 1minutes and washed with clean water
- ❖ The smear was decolorized with few drops of alcohol solution and was immediately washed off using clean water.
- ❖ The smear was then counter stained with safranin solution for about 2 minutes and wash off with a running tap.
- ❖ The stained smear was allowed to air dry, after which a drop of oil immersion was placed on it and examined with x100 objective lens.

After gram staining of the culture on the plates, further biochemical test were carried out on the organisms isolated for further identification.

Sugar Fermentation Test

This was done to investigate the ability of isolates to utilize sugar. The following sugars were used: glucose, manitol, sucrose and lactose. One gram (1g) of each sugar was dissolved in 10ml of water in different test tubes. One point five gram (1.5g) of bacteriological peptone and 1ml of bromo-thimoblue tubes. The mixture was dispensed into test tubes, sterilized by autoclaving with inverted Durham tubes at 115°C for 10 minutes and incubated for 24 hours at 37°C. Change of color from blue to yellow indicated positive test result, which also signifies the production of acid. Presence of air bubbles at the top of the Durham tube indicated gas production and absence of air bubbles indicated no gas production.

Standardization of Test Bacteria

The test organisms were standardized by using a sterile wire loop, to pick 3–5 pure cultures of the test micro organism and emulsified in 3–4 ml of sterile physiological saline. The turbidity reading of the 0.5 McFarland Standard was recorded as Absorbance in a Spectrophotometer at 540 nm, while the turbidities of the test organisms were adjusted to match the absorbance of the 0.5 McFarland standard at the same wave length, using physiological saline. NB: 0.5 McFarland contains 1.5×10^8 cfu/ml.

Antimicrobial Susceptibility Test

The antibacterial activities of the extracts against the test bacteria were evaluated by modified disc diffusion methods (Chessbrough, 1984). Exactly 25 µl of 0.5 McFarland standardized suspension of tes bacteria (1.5×10^8 cfu ml⁻¹) were cultured onto the Mueller-Hinton plates by pour plate method. Exactly 50 µl of the extracts were used to impregnate the 6mm filter paper discs and placed on two portions of the agar plate. The Inhibition zone diameters of the various plates were measured and recorded in millimeters. All experiments were done in triplicates. Negative controls were set up with sterile physiological saline and positive controls were set up using 50 µg/ml Ciprofloxacin for bacteria (Agu *et al.*, 2013; Awah *et al.*, 2016; Adindu *et al.*, 2016; Awah *et al.*, 2017; Ubaoji *et al.*, 2020)

Results

The bacteria isolated from the meat samples include: *Escherichia* spp, *Staphylococcus* spp and *Salmonella* spp. The total viable counts of the samples are shown in table 4.1. The results of their individual biochemical reactions are shown in table.

Total viable Count (cfu/ml)

Meat Samples	Total Viable Count (cfu/g)	
	NA	MA
A	4.5 x 10 ⁵	3.7 x 10 ⁵
B	3.1 x 10 ⁵	3.0 x 10 ⁵
C	3.5 x 10 ⁵	2.7 x 10 ⁵
D	2.7 x 10 ⁵	2.5 x 10 ⁵

Key: NA = Nutrient Agar, MA = MacConkey Agar

Table 4.2: Characteristics of Bacterial Isolates

COLONY MORPHOLOGY	Gram Reaction									SUGAR FERMENTATION				Probable Organism
		Motility	Catalase	Coagulase	Indole	Methyl Red	Citrate	Oxidase	Glucose	Maltose	Lactose	Mannitol		
Yellowish,, Moist and Smooth Colonies	+ Cocci	-	+	+	-	+	+	-	A	A	A	A	<i>Staphylococcus</i> spp	
Appear Pink or Red in MacConkey Agar	-Rod	-	+	-	-	+	+	-	AG	A	AG	AG	<i>E. coli</i>	
Yellowish, Moist and Smooth Colonies	+ Cocci	+	+	-	+	+	-	-	A	A	A	A	<i>Staphylococcus</i> spp	
Appear Milkish in Nutrient Agar	-Rod	-	+	+	-	-	+	-	A	A	-	-	<i>Salmonella</i> spp	

KEY: (+) = Positive
 (-) = Negative
 AG = Acid and Gas
 A = Acid

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Discussion

Four (4) fresh meat samples were collected from different vendors from Afor Oba Market, Anambra State. This result of this study shows the following bacteria isolated from the samples which include: *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. The total viable count ranged between 2.7 x 10⁵ to 4.5 x 10⁵ on Nutrient Agar, while in MacConkey the total viable count ranged between 2.5 x 10⁵ to 3.7 x 10⁵. The presence of this bacteria isolate could be as a result of poor hygienic practices, unsterilized container used in storage of the meat, unsterilized materials used in preparation of the meat for selling. The isolated bacteria are in line with the findings of Abouzeed *et al.*, (2020) who isolated *Escherichia coli*, and staphylococcus on meat samples sold in Kaduna Market Metropolis. The result of this study showed that the bacterial count varied with location and this may be due to differences in management practices resulting in different level of organic loads in the fresh meat (Adams *et al.*, 2004). The source of microorganism could also be traced to source of contaminated water used in washing the meat after killing of the animal or poor hygienic slaughtering ground.

The recovery of *Escherichia coli* in the sample could suggest a possible faecal contamination of the water. The faecal material may be as a result of flood dirt with animal's manure which is discharged directly into the water used in washing the meat at slaughtering ground (Wood, 2003). Antibiotic sensitivity test was conducted and the following antibiotics names were recorded sensitive to the isolate *E. coli* with Chloraphenical having 14mm, Ciprofloxacin 18mm, Gentamycin having 21mm, Agabaxin 22mm, Zolacin 22mm, Tetracyclin 2mm, and was resistant to Amoxicillin and Seprtrin. The antibiotic names were recorded sensitive to the isolate *Staphylococcus* with Gentamycin having 3mm, Nitroforatoin 9mm, Cotrimacol 6mm, Oflcacin 6m, Oerfuroxine 1mm, Cephalixin 4mm, tetracycline 2mm.

The findings of this study are also in line with the findings of Ateba *et al.*, (2011) on the investigation into microorganisms associated with fresh meat sold in Odogolo Market Mina, Niger State. The various bacteria obtained from the study constitute part of the normal flora of the digestive system of human and animal when taken and hence may be found in their waste product, they may also be present in water, soil, plant materials and atmosphere.

Conclusion

The fresh meat samples according to the result of the investigation clearly signify that they are not of the same microbial quality. These differences may be due to improper sanitary conditions during slaughtering or storage. The presence of these pathogenic bacteria is thus an indication of microbial contamination to the health of man. More importantly, during processing high level of cleanliness should be maintained to ensure that the products are of good quality.

Recommendations

1. Strict hygienic practices must be followed during slaughtering, cutting and storage of fresh meat.
2. The authority should issue the license to small dairy producers after the assurance of minimum level of good manifesting practice.

REFERENCES

- Abouzeed, Y.M., Hariharan,H.,Poppe, C. and Kibenge, F.S.B. (2020). Characterization of Salmonella isolates from beef cattle, broiler chicken and human sources on Prince Edward Island. *Comparative Immunology, Microbiology and Infectious Diseases* **23**:253 – 266.
- Adindu, C.S., Odili, L.C., Nwagu, E.E., Agu, K.C., Awah, N.S., Okeke, B.C. and Orjionwe, R.N. (2016). Phytochemical and Antimicrobial Screening of Cola gigantea Leaves, Stem and Bark. *Universal Journal of Microbiology Research*, 4 (2): 49-54
- Agu, K.C., Archibong, E. J., Anekwe, D.C, Ago, C.A., Okafor, A.C. and Awah, N.S. (2014). Assessment of Bacteria Present in Yoghurt Sold on Awka Metropolis. *Scholars Journal of Applied Medical Sciences*, 2 (6D): 3071-3075.
- Agu, K.C., Igweoha, C.A. and Umeh, C.N. (2013). Antimicrobial Activity of the Ethanolic and Petroleum Ether Extracts of Tangerine Seed on Selected Bacteria. *International Journal of Agriculture and Bioscience*, 2 (1): 22-24.
- Agu, K.C., Ukponmwan, I.O., Orji, M.U., Awah, N.S., Anaso, C.I. and Udemezue, O.I. (2013). Prevalence of Pathogenic Bacteria in Smoked Fish sold in Major Retail Markets in Benin, Nigeria. *International Journal of Applied Science and Engineering*, 1 (1): 1- 4.
- Agu, K.C., Ikwuka, O.I., Umeoduagu, N.D., Victor-Aduloju A.T., Uwanta L.I., Ikenwa, B.O., Nwiyi, I.U., Chidubem-Nwachinemere, N.O., Nwosu, J.C. and Okafor, I.J. (2023). Fermentation and Production of Cocoyam (*Colocasia esculenta*) Flour Fortified with Soybean Powder. *Cognizance Journal of Multidisciplinary Studies*, 3 (6): 449-470. DOI: 10.47760/cognizance.2023.v03i06.031
- Anaukwu, C.G., Nwangwu, F.C., Okafor, I.O., Ezemba, C.C. Orji, C.C., Agu, K.C. and Archibong, J.E. (2015). Microbiological Analysis of Burukutu beverage produced in southern part of Nigeria. *European Journal of Experimental Biology*, 5(8):18-22
- Anaukwu, C.G., Ugwuoke, G.O., Ekwealor, I.A., Okafor, O.C. and **Agu, K.C.** (2015). Preliminary Study of Bacterial Isolates from Indigenous Ready – To – Eat Salad Vegetables. *American Journal of Life Science Researches*, (3) 4: 282-286
- Awah, N.S., **Agu, K.C.***, Ikedinma, J.C., Uzoechi, A.N., Eneite, H.C., Victor-Aduloju A.T., Umeoduagu, N.D., Onwuatuogwu, J.T.C., Ilikannu S.O. (2017). Antibacterial Activities of the Aqueous and Ethanolic Extracts of the Male and Female *Carica papaya* Leaves on Some Pathogenic Bacteria. *Bioengineering and Bioscience*, 5(2): 25-29. DOI: 10.13189/bb.2017.050201
- Awah, N.S.* , **Agu, K.C.***, Muokwe, J., Irondi, C., Okeke, C.B., Anaukwu, C.G., Archibong, E.J., Iloanus, C.A., Ngenegbo, U. and Umeoduagu, N. (2016). Microbial Assessment of Yoghurts Sold in Amawbia, Nigeria. *Universal Journal of Microbiology Research*, 4 (2): 55-58.
- Awah, N. S., Agu, K. C., Okorie, C. C., Okeke, C. B., Iloanus, C. A., Irondi, C. R., Udemezue, O. I., Kyrian-Ogbonna, A. E., Anaukwu, C. G., Eneite, H. C., Ifediegwu M. C., Umeoduagu, N. D., Abah, N. H. and Ekong, U. S. (2016). In-vitro Assessment of the Antibacterial Quality of Some Commonly Used Herbal and Non-herbal Toothpastes on Streptococcus mutans. *Open Journal of Dentistry and Oral Medicine* 4(2): 21-25, <http://www.hrpub.org/DOI:10.13189/ojdom.2016.040>

- Banerjee, R., Kapoor, K.N., Agarwal, R.K. and Ghatak, S. (2001). Verotoxin producing Escherichia coli (VETO) in foods of animal origin. *Journal Food Science and Technology***38**: 82 – 84.
- Bhandare, S.G., Sherikar, A.T.,Paturkar, A.M.,Waskar,V.S. and Zende, R.J.(2007). A comparison of microbial contamination of sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. *Food Control***18**:854 – 868.
- Enujiugha VN, 2005. Quality dynamics in the processing of underutilized legumes and oil seeds. In: Crops, Growth, quality and biotechnology (R. Dris, ed.), WFL publishers, Helsinki, Finland, Pp: 732-746.
- Mbachu A.E., Etok , C.A., **Agu, K.C***, Okafor, O.I., Awah, N.S., Chidi-Onuorah, L.C., Ekwueme, V.C., Okpala, J., Ogbue, M.O. and Ikele, M.O. (2014). Microbial Quality of Kunu Drink sold in Calabar, Cross River State, Nigeria. *Journal of Global Biosciences*, **3** (2): 511-515.
- Niyonzima, E., Borai, D. and Ongoli, M.P. (2013). Assessment of beef meat microbial contamination during skinning, dressing, transportation and marketing at a commercial abattoir in Kigali city, Rwanda. *Pakistan Journal of Food Sciences* **23**(3): 133 – 138.
- Niyonzima, E., Ongol, M. P., Kimonyo, A. and Sindic, M. (2015). Risk factors and control measures for bacterial contamination in the bovine meat chain: A Review on Salmonella and Pathogenic E. coli. *Journal of Food Research***4**(5):0887 – 0895.
- Okpalla J., Ubajekwe, C.C., **Agu, K.C.**and Iheukwumere, I. (2012). Biochemical Changes of Melon Seeds (*Citrullus vulgaris*) Fermented by pure cultures of *Bacillus licheniformis*. *International Journal of Agriculture and Bioscience*. **1** (1): 42- 45
- Scanga, J.A., Grona, A.D., Belk, K.E., Sofos, J.N., Bellinger G.R. and Smith, G.C. (2000). Microbiological contamination of raw beef trimmings and ground beef. *Meat Science***56**: 145 – 152.
- Ubaaji, K., Nwosu, O., Agu, K., Nwozor, K., Ifedilichukwu, N., & Okaka, A. (2020). Gas Chromatographic Analysis of the Phyto-Constituents and the Assessment of the Anti-Microbial Properties of the Leave Extracts of Nigeria-Grown Ginkgo biloba. *Journal of Scientific Research in Medical and Biological Sciences*, *1*(2), 45-56. <https://doi.org/10.47631/jsrmb.v1i2.57>