

# Bacteriological Profile of Leafy Vegetables Sold in Agbor Market, Delta State, Nigeria

\*Sandra I. Uwagboi and Thelma E. Konyeme

Department of Biological Sciences, University of Delta, Agbor, Delta State, Nigeria

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## ABSTRACT

**Background:** This study investigated the bacterial contamination of selected leafy vegetables sold in Edike Market, Agbor, Delta State, Nigeria. This study establishes the health risks posed by open-air market contamination on the local leafy vegetables and assess specific gaps in the region's food safety status.

**Methods:** Five vegetable types *Telfairia occidentalis*, *Celosia argentea*, *Albizia zygia*, were analyzed using standard microbiological techniques for total heterotrophic bacterial count (THBC), isolation, and identification of bacterial species.

**Results:** The THBC ranged from  $3.6 \times 10^7$  CFU/g in *Celosia argentea* to  $1.85 \times 10^8$  CFU/g in *Gongronema latifolium*, indicating heavy microbial contamination of all samples. Bacterial isolates recovered included *Escherichia coli*, *Staphylococcus* spp., *Bacillus cereus*, *Salmonella* spp., and *Klebsiella* spp. *Escherichia coli* was the most prevalent isolate, occurring in all vegetable samples (100% distribution) and accounting for 36.0% of total isolates, while *Klebsiella* spp. was the least prevalent (8.0%). *Gongronema latifolium* harbored the highest diversity of bacterial isolates (80%), whereas *Myrianthus arboreus* had the lowest (40%).

**Conclusion:** The findings imply that vegetables sold in the study area are exposed to significant contamination during cultivation, handling, transportation, and marketing, posing potential public health risks. Improved hygiene practices, proper washing of vegetables, and enhanced market sanitation are recommended to reduce the risk of foodborne infections associated with vegetable consumption.

**Keywords:** Bacterial contamination, Food safety, Leafy vegetables, Microbial quality, Public health

## 1.0 INTRODUCTION

Vegetables constitute an essential component of human nutrition, providing vital vitamins, minerals, dietary fiber, and numerous bioactive compounds that contribute to health promotion and the prevention of chronic diseases [1, 2]. In many developing countries, including Nigeria, vegetables form a significant part of the daily diet and are widely consumed due to their affordability and nutritional value. Common vegetables marketed in Nigeria include leafy vegetables such as fluted pumpkin (*Telfairia occidentalis*) and spinach (*Amaranthus* spp.), root vegetables such as carrots, and fruit vegetables such as tomatoes and cucumbers [3]. These products are predominantly sold through open-air markets, which serve as major distribution centers for fresh produce and cater to diverse socio-economic populations. Despite their nutritional importance, vegetables are highly susceptible to microbial contamination throughout the production and distribution chain. The microbiological safety of vegetables has become a global public health concern due to the increasing incidence of foodborne diseases and the growing emergence of antimicrobial-resistant bacterial pathogens [4]. Contamination may occur at multiple stages, including cultivation, harvesting, transportation, storage, marketing, and handling by vendors and consumers [5, 6]. During cultivation, the use of contaminated irrigation water, untreated animal manure, and exposure to polluted soils can introduce pathogenic

\*Corresponding author: Email: [Sandra.uwagboi@unidel.edu.ng](mailto:Sandra.uwagboi@unidel.edu.ng); Phone: +2348145184658

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# Uwagboi and Konyeme: Bacteriological Profile of Leafy Vegetables Sold in Agbor Market, Delta State, Nigeria

microorganisms onto vegetable surfaces. Post-harvest contamination may occur through improper handling, poor sanitation practices, contaminated storage facilities, and unhygienic market environments [7]. The risk of microbial contamination is particularly pronounced in tropical regions such as Nigeria, where warm temperatures and high humidity create favorable conditions for microbial growth and survival. These environmental conditions not only accelerate vegetable spoilage but also enhance the proliferation of pathogenic microorganisms capable of causing foodborne illnesses [8]. Open-air markets, which often lack adequate sanitation facilities, proper waste disposal systems, and effective temperature control measures, further increase the likelihood of contamination. Consequently, vegetables sold in such environments may serve as vehicles for the transmission of pathogenic bacteria to consumers. Leafy vegetables are especially vulnerable to microbial contamination because of their large surface area, high moisture content, and complex leaf structures, which facilitate microbial attachment and persistence. Numerous outbreaks of foodborne diseases associated with the consumption of contaminated vegetables have been reported worldwide. According to the Food and Agriculture Organization and the World Health Organization [9], leafy vegetables accounted for approximately 16%, 18%, 12%, 13%, and 5% of foodborne outbreaks in Australia, Brazil, Canada, Finland, and Sweden, respectively, between 1996 and 2006. Similarly, in the United States, approximately 78% of 501 foodborne disease outbreaks recorded between 1998 and 2012 were associated with salad vegetables [10]. In Africa, contaminated vegetables have also been implicated in cholera outbreaks in Zambia [11] and bacterial infections in Ghana [12], highlighting the public health significance of vegetable-borne pathogens across the continent. Several studies conducted in Nigeria have documented the presence of diverse microbial contaminants on vegetables sold in local markets. These contaminants include *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp., as well as fungal species such as *Candida* spp. and *Aspergillus* spp. [13, 14]. Ade Uzeh *et al.* [15] reported the occurrence of *Staphylococcus aureus* in carrots, cucumbers, cabbage, and lettuce sold in markets within Lagos State. Similarly, Eni *et al.* [13] detected *Staphylococcus* spp. and *Streptococcus* spp. on fruits marketed in Ilorin, while *E. coli* contamination has been reported in lettuce, apples, coleslaw, and melons [16]. The presence of these microorganisms on vegetables is of considerable concern because many are recognized causes of foodborne diseases and opportunistic infections in humans [17]. An emerging challenge associated with bacterial contamination of food products is antimicrobial resistance (AMR). The widespread occurrence of antibiotic-resistant bacteria in foods has become a major global health concern because resistant pathogens can compromise the effectiveness of treatment and increase disease severity, hospitalization rates, and mortality. In Nigeria, antibiotic-resistant strains of *E. coli*, *Salmonella* spp., and *Staphylococcus aureus* have been isolated from various food products, indicating that fresh produce may serve as reservoirs and transmission routes for resistant microorganisms [18]. Furthermore, studies conducted in Delta State have reported high bacterial loads and significant antibiotic resistance among bacterial isolates recovered from poultry and fish feeds sold in Abraka, suggesting the potential for similar contamination risks in vegetables marketed within nearby communities such as Agbor [19]. Given the nutritional importance of vegetables and the potential health risks associated with microbial contamination and antimicrobial resistance, continuous monitoring of the microbiological quality of vegetables sold in local markets is essential. Such investigations provide valuable information on the prevalence of pathogenic bacteria, the level of contamination, and the antimicrobial susceptibility patterns of isolates. The findings can contribute to the development of effective food safety interventions, improved market sanitation practices, and public health policies aimed at reducing the burden of foodborne diseases associated with vegetable consumption. Therefore, this study aimed to isolate and identify bacterial contaminants associated with selected leafy vegetables sold in Edike Market, Agbor, Delta State, Nigeria.

## 2.0 MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Biological materials

Biological materials used for this study were: *Telfairia occidentalis*, *Celosia argentea*, *Gongronema latifolium*, *Myrianthus arboreus* and *Albizia zygia*.

#### 2.1.2 Chemicals and reagents

Chemicals and reagents used for this study were: Nutrient agar, MacConkey agar, Simmons citrate agar, Crystal violet, Safranin and Hydrogen peroxide.

#### 2.1.3 Equipment



The following equipment were used for this study: Incubator, Autoclave, Microscope, Analytical balance and Hot air oven.

## 2.2 Methods

### 2.2.1 Study Area

This study was conducted at Edike Market, a major open-air market located in Agbor, the administrative headquarters of Ika South Local Government Area, Delta State, Nigeria. Agbor is situated at approximately 6°15'13.50"N latitude and 6°11'39.12"E longitude within the humid rainforest zone of southern Nigeria. The area experiences a tropical climate characterized by high temperatures, elevated humidity, and substantial rainfall throughout most of the year. Edike Market serves as an important commercial center for the sale and distribution of agricultural products, including a wide variety of fresh vegetables sourced from neighboring farming communities. The warm and humid environmental conditions prevalent in the region provide favorable conditions for the growth and survival of microorganisms, particularly on highly perishable food items such as vegetables. The market was selected for this study because of its high volume of commercial activities, large number of vegetable vendors and consumers, and its strategic role in the local vegetable supply chain. Furthermore, the predominantly open-market structure and limited storage facilities commonly observed within the market may increase the susceptibility of vegetables to microbial contamination and spoilage, making it an appropriate location for investigating bacterial contamination of vegetables.

### 2.2.2 Sampling Procedure

Five commonly consumed leafy vegetables, namely pumpkin leaves (*Telfairia occidentalis*), shoko leaves (*Celosia argentea*), ujuju leaves (*Myrianthus arboreus*), utazi leaves (*Gongronema latifolium*), and onunuagbon leaves (*Albizia zygia*), were randomly purchased from different vendors at Edike Market, Agbor, Delta State, Nigeria. Each sample was aseptically collected into sterile, labeled polyethylene bags to prevent cross-contamination. The samples were transported in an ice-packed cooler to the Microbiology Laboratory for analysis. All samples were processed within 6 h of collection.

### 2.2.3 Sterilization of Materials and Media Preparation

Glassware, including Petri dishes, test tubes, conical flasks, measuring cylinders, pipettes, and McCartney bottles, were thoroughly washed, dried, wrapped in aluminum foil, and sterilized in a hot-air oven at 160°C for 1 h. Work surfaces were disinfected with 70% ethanol, and aseptic conditions were maintained using a Bunsen burner flame. Culture media, including Nutrient Agar (NA), MacConkey Agar (MAC), and Simmons Citrate Agar, were prepared according to the manufacturers' instructions and sterilized by autoclaving at 121°C for 15 min. Prepared media and biochemical reagents were stored at 4°C until use.

### 2.2.4 Isolation and Enumeration of Bacteria

Approximately 1 g of each vegetable sample was aseptically weighed and homogenized in 9 mL of sterile normal saline to obtain a stock suspension. Serial ten-fold dilutions were prepared up to 10<sup>-4</sup>. Aliquots (1 mL) from the appropriate dilution (10<sup>-2</sup>) were inoculated into sterile Petri dishes using the pour plate technique. Molten Nutrient Agar cooled to approximately 45°C was added and mixed gently before allowing the medium to solidify. The inoculated plates were incubated at 37°C for 24 h in an inverted position. After incubation, colonies on countable plates (30–300 colonies) were enumerated and expressed as colony-forming units per gram (CFU/g) using the formula:

$$\text{CFU/g} = \text{Number of colonies} \times \text{Dilution factor} / \text{Volume plated}$$

### 2.2.5 Purification of Bacterial Isolates

Distinct bacterial colonies were selected and purified by repeated streaking on Nutrient Agar plates. Pure cultures obtained were maintained on Nutrient Agar slants and incubated at 37°C for 24 h before further characterization.

### 2.2.6 Characterization and Identification of Bacterial Isolates

Bacterial isolates were initially characterized based on colony morphology, including shape, size, elevation, margin, pigmentation, and surface appearance. Further identification was carried out using Gram staining and standard biochemical tests according to the methods described by [20].



# Uwagboi and Konyeme: Bacteriological Profile of Leafy Vegetables Sold in Agbor Market, Delta State, Nigeria

## 2.2.6.1 Gram Staining

Pure bacterial cultures were smeared on clean grease-free slides, heat-fixed, and stained with crystal violet for 1 min. The slides were rinsed with distilled water, treated with Gram's iodine for 1 min, decolorized with 95% ethanol for 30 s, and counterstained with safranin for 1 min. After air-drying, the stained preparations were examined microscopically under oil immersion.

## 2.2.6.2 Catalase Test

A small portion of each bacterial isolate was placed on a clean glass slide, and a drop of freshly prepared 3% hydrogen peroxide was added. The production of gas bubbles indicated a positive catalase reaction.

## 2.2.6.3 Simmons Citrate Test

Bacterial isolates were inoculated onto Simmons Citrate Agar slants and incubated at 37°C for 24–48 h. Utilization of citrate as the sole carbon source was indicated by growth and a color change of the medium from green to blue, while no color change indicated a negative reaction.

## 2.2.6.4 Oxidase Test

The oxidase test was performed using freshly prepared oxidase reagent (1% tetramethyl-p-phenylenediamine dihydrochloride). A colony of the test organism was smeared onto reagent-soaked filter paper. The development of a dark purple or blue color within 30 s was recorded as a positive oxidase reaction.

## 2.2.6.5 Urease Test

Urease activity was determined using Christensen's Urea Agar slants. Pure cultures were inoculated onto the slants and incubated at 37°C for 24–48 h. A change in the medium color from yellow-orange to pink indicated urease production, while no color change was recorded as a negative reaction.

## 2.3 Statistical analysis

Data are presented as mean  $\pm$  standard error (SE) from five replicates. Biostatistical analysis was performed using SPSS version 24 alongside a descriptive statistical approach. A one-way Analysis of Variance (ANOVA) was used to determine the variations among the parameters, and p-value of less than 0.05 was considered statistically significant.

## 3. RESULT

The total heterotrophic bacterial counts varied significantly ( $P < 0.05$ ) among the vegetable samples. *Gongronema latifolium* (Utazi leaves) recorded the highest bacterial load ( $1.85 \times 10^8$  CFU/g), while *Celosia argentea* (Shoko leaves) had the lowest maximum count ( $3.6 \times 10^7$  CFU/g). Several plates from *Telfairia occidentalis* and *Gongronema latifolium* produced colonies that were too numerous to count (TNC), all vegetable samples harbored high bacterial populations.

Table 1: Total Heterotrophic Bacterial Counts (CFU/g) of Vegetable Samples

Vegetable Sample	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
<i>Celosia argentea</i> (Ca)	$1.56 \times 10^4$	$1.20 \times 10^5$	$7.8 \times 10^5$	$7.0 \times 10^6$	$3.6 \times 10^7$
<i>Telfairia occidentalis</i> (To)	TNC	$1.70 \times 10^5$	$8.2 \times 10^5$	$7.0 \times 10^6$	$3.8 \times 10^7$
<i>Myrianthus arboreus</i> (Ma)	$1.60 \times 10^4$	$1.40 \times 10^5$	$8.2 \times 10^5$	$7.0 \times 10^6$	$6.8 \times 10^7$
<i>Albizia zygia</i> (Az)	$1.58 \times 10^4$	$1.08 \times 10^5$	$1.00 \times 10^6$	$8.0 \times 10^6$	$6.8 \times 10^7$
<i>Gongronema latifolium</i> (Gl)	TNC	TNC	$3.07 \times 10^6$	$2.30 \times 10^7$	$1.85 \times 10^8$

Key: TNC = Too Numerous to Count CFU/g = Colony Forming Units per Gram

Table 2: Recategorization showing maximum heterotrophic bacterial count recorded for each vegetable:

Vegetable Sample	Maximum Bacterial Count (CFU/g)
<i>Gongronema latifolium</i>	$1.85 \times 10^8$
<i>Myrianthus arboreus</i>	$6.8 \times 10^7$
<i>Albizia zygia</i>	$6.8 \times 10^7$
<i>Telfairia occidentalis</i>	$3.8 \times 10^7$
<i>Celosia argentea</i>	$3.6 \times 10^7$

Maximum bacterial count representation from Table 1



### 3.1 Percentage distribution

*Escherichia coli* was the most prevalent bacterial isolate, significantly ( $P < 0.05$ ) occurring in all five vegetable samples and accounting for 100% distribution. *Bacillus cereus*, *Salmonella* spp., and *Staphylococcus* spp. each occurred in three of the five vegetable samples, representing 60% distribution. *Klebsiella* spp. was the least prevalent isolate, occurring only in *Gongronema latifolium* (Utazi leaves), with a distribution of 20%. Among the vegetables, Utazi leaves harbored the highest diversity of bacterial isolates (80%), while Ujuju leaves recorded the lowest bacterial diversity (40%).

Table 3: Percentage Distribution of Bacterial Isolates in Vegetable Samples

Bacterial Isolate	Number of Positive Samples	Percentage Distribution (%)
<i>Escherichia coli</i>	5	100.0±0.60
<i>Bacillus cereus</i>	3	60.0±0.21
<i>Salmonella</i> spp.	3	60.0±0.45
<i>Staphylococcus</i> spp.	3	60.0±0.33
<i>Klebsiella</i> spp.	1	20.0±0.42

Mean ± Standard error

Table 4: Percentage Distribution of Bacterial Isolates across Vegetable types

Vegetable Sample	Number of Isolates Detected	Percentage Occurrence (%)
Pumpkin ( <i>Telfairia occidentalis</i> )	3	60.0±0.33
Shoko ( <i>Celosia argentea</i> )	3	60.0±0.20
Ujuju ( <i>Myrianthus arboreus</i> )	2	40.0±0.62
Utazi ( <i>Gongronema latifolium</i> )	4	80.0±0.35
Onunuagbon ( <i>Albizia zygia</i> )	3	60.0±0.21

Mean ± Standard error

### 3.2 Prevalence of Bacterial Isolates from Vegetable Samples

A total of 25 bacterial isolates were recovered from the vegetable samples examined. The distribution of the isolates showed that *Escherichia coli* was the most prevalent bacterium ( $P < 0.05$ ), accounting for 9 isolates (36.00%). This was followed by *Staphylococcus* spp., which constituted 6 isolates (24.00%), and *Bacillus cereus*, with 5 isolates (20.00%). *Salmonella* spp. accounted for 3 isolates (12.00%), while *Klebsiella* spp. was the least frequently isolated organism, representing 2 isolates (8.00%).

Table 5: Percentage Prevalence

Suspected Organism	Number Present	Percentage Prevalence (%)
<i>Bacillus cereus</i>	5	20.00
<i>Escherichia coli</i>	9	36.00
<i>Salmonella</i> spp.	3	12.00
<i>Klebsiella</i> spp.	2	8.00
<i>Staphylococcus</i> spp.	6	24.00

## 4.0 DISCUSSION

The results of this study revealed high levels of bacterial contamination in all vegetable samples examined, indicating that fresh vegetables sold in Edike Market, Agbor, may serve as potential vehicles for the transmission of foodborne pathogens. The total heterotrophic bacterial counts (THBC) ranged from  $3.6 \times 10^7$  CFU/g in *Celosia argentea* to  $1.85 \times 10^8$  CFU/g in *Gongronema latifolium* (Utazi). These values are considerably high and showing significant microbial contamination of vegetables. This result is understandable since the high bacterial loads observed may be attributed to contamination occurring at various stages of production down to the end consumer. This finding corroborated with [21], who reported that the contamination of vegetable emerges from various stages of production and distribution



## Uwagboi and Konyeme: Bacteriological Profile of Leafy Vegetables Sold in Agbor Market, Delta State, Nigeria

including cultivation with contaminated irrigation water, application of untreated organic manure, poor handling practices during harvesting, transportation, and exposure to unsanitary market conditions [5, 21]. This also agrees with the result of [5], who reported that the major contamination recorded emanated from untreated organic manure used in implementing organic farming process. The exceptionally high bacterial count recorded in *Gongronema latifolium*. This variation could be attributed to its leaf morphology and handling practices. Conversely, Leafy vegetables possess large surface areas and numerous folds that facilitate the attachment and survival of microorganisms. These results corroborated with [7], who reported that vegetables displayed openly in markets are frequently exposed to dust, insects, contaminated water, and repeated handling by vendors and consumers, all of which can contribute to increased microbial loads. The occurrence of plates that were too numerous to count (TNC) for *Telfairia occidentalis* and *Gongronema latifolium* further emphasizes the heavy microbial burden associated with these vegetables. Similar high bacterial counts have been reported in vegetables sold in Nigerian markets by [13, 14], and [15], who attributed such contamination to inadequate sanitary conditions during marketing and storage. The occurrence and distribution of bacterial isolates revealed that *Escherichia coli* was present in all vegetable samples, giving a distribution frequency of 100%. The widespread occurrence of *E. coli* is of particular public health concern because it is commonly regarded as an indicator of fecal contamination. Its high presence could be due to possible contamination from animal manure, sewage-polluted irrigation water, contaminated soil, or poor personal hygiene among handlers [4, 5]. Similar findings have been reported by [13] and [22], who isolated *E. coli* from vegetables sold in Nigerian and Ghanaian markets, respectively. The dominance of *E. coli* in the present study highlights the potential risk of gastrointestinal infections associated with the consumption of raw or inadequately washed vegetables. *Bacillus cereus*, *Salmonella* spp., and *Staphylococcus* spp. each exhibited a distribution frequency of 60%, indicating their widespread occurrence among the vegetable samples. The presence of *Bacillus cereus* may be attributed to its natural occurrence in soil and its ability to produce highly resistant spores that can survive adverse environmental conditions. This finding on the prevalence of *Bacillus cereus* was reported by [23], saying that vegetables are cultivated in direct contact with soil, contamination by *Bacillus* species is expected. Consumption of foods contaminated with *B. cereus* has been associated with emetic and diarrheal food poisoning syndromes [23]. The isolation of *Salmonella* spp. from 60% of the vegetable samples is noteworthy because members of this genus are among the most important causes of foodborne gastroenteritis worldwide. The occurrence of *Salmonella* could be attributed to the untreated organic manure used. This agrees with [23], who reported that contamination of soil by animal feces, untreated manure, contaminated irrigation water, or poor sanitary practices during handling and marketing is responsible for *Salmonella* prevalence. This also agrees with the report of [9]. The detection of this pathogen on vegetables intended for direct consumption poses a significant health risk, particularly in developing countries where food safety monitoring may be inadequate. Similarly, the recovery of *Staphylococcus* spp. from three of the five vegetable types suggests contamination from human sources. *Staphylococcus aureus* is commonly found on the skin, nasal passages, and hands of healthy individuals and can easily be transferred to food during harvesting, transportation, processing, or sale [20]. The occurrence of this organism therefore reflects poor personal hygiene among handlers and vendors. Comparable findings were reported by [15], who isolated *Staphylococcus aureus* from various vegetables sold in Lagos markets. *Klebsiella* spp. was the least prevalent isolate, occurring in only one vegetable sample and accounting for 20% distribution. Although its occurrence was relatively low, the presence of *Klebsiella* spp. remains significant because members of this genus are opportunistic pathogens associated with urinary tract infections, pneumonia, septicemia, and other nosocomial infections [17]. Their presence may indicate environmental contamination from soil, water, or human activities. The analysis of bacterial diversity across vegetable types showed that *Gongronema latifolium* harbored the highest diversity of bacterial isolates (80%), while *Myrianthus arboreus* recorded the lowest diversity (40%). The higher diversity observed in *Gongronema latifolium* may be associated with its broad leaf surface and greater exposure to contamination sources during cultivation and marketing. Differences in bacterial diversity among vegetables may also be influenced by plant morphology, moisture content, surface characteristics, and storage conditions [6]. The prevalence analysis further demonstrated that *Escherichia coli* was the dominant bacterial isolate, accounting for 36.0% of all isolates recovered. This was followed by *Staphylococcus* spp. (24.0%), *Bacillus cereus* (20.0%), *Salmonella* spp. (12.0%), and *Klebsiella* spp. (8.0%). The predominance of *E. coli* corroborates previous studies conducted in Nigeria and other developing countries, where fecal contamination has been identified as a major source of vegetable-associated bacterial contamination [13, 14]. The detection of multiple pathogenic bacterial species further suggests that vegetables sold in the study area may constitute an important reservoir of foodborne pathogens.

### 5. CONCLUSION

This study demonstrated that leafy vegetables sold in Edike Market, Agbor, are heavily contaminated with heterotrophic and potentially pathogenic bacteria. All vegetable samples examined contained high bacterial loads, with



*Gongronema latifolium* recording the highest contamination level. The isolation of *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp., *Bacillus cereus*, and *Klebsiella* spp. indicates possible fecal, environmental, and human-source contamination of the vegetables. The predominance of *Escherichia coli* suggests poor sanitary conditions during production and marketing and highlights the potential risk of foodborne disease transmission through consumption of raw or inadequately washed vegetables.

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#### **Authors contributions**

Mrs Sandra I. Uwagboi conceptualized the work, did the laboratory analysis and wrote the manuscript. Dr. Thelma E. Konyeme conducted the sample collection and processing. All authors read and approved the manuscripts.

#### **Ethical Requirements**

The plant samples were authenticated at the herbarium unit by a taxonomic expert in the Department of Biological Sciences, University of Delta, Agbor, Delta State, Nigeria

#### **Conflict of interest**

The authors declared no conflict of interest.

#### **AI Disclosure**

Generative artificial intelligence tools were not used in the preparation of this manuscript.

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## Uwagboi and Konyeme: Bacteriological Profile of Leafy Vegetables Sold in Agbor Market, Delta State, Nigeria

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