
Microbiological Load of Pumpkin (Ugu) Leaves (*Telfairia occidentalis*) Sold at Eke Okigwe Market Imo State, Nigeria

Victor Maduabuchukwu Ezeakonamba*¹, Gabriel C. Ezeakonamba*² and
Christiana C. Ezeakonamba*³

¹Department of Microbiology, Abia State University, P.M.B. 2000, Uturu, Abia State, Nigeria.

²Department of Microbiology, Imo State University, P.M.B. 2000, Owerri, Imo State, Nigeria.

³Department of Microbiology, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria.

Corresponding Author: Victor Ezeakonamba, Email: Vickchi844@gmail.com

Abstract

Pumpkin leaves (*Telfairia occidentalis*) are widely consumed in southeastern Nigeria and often sold in informal markets with minimal hygiene practices. This study assessed the microbial quality of pumpkin leaves sold at five locations within the Okigwe main market, Imo State. Thirty samples were randomly collected and analyzed using standard microbiological techniques to determine bacterial and fungal loads, identify microbial species, and assess antibiotic susceptibility. Bacterial counts ranged from 3.6×10^5 to 7.3×10^5 CFU/g and fungal counts from 1.5×10^3 to 5.6×10^3 CFU/g. Pathogenic organisms including *Escherichia coli*, *Salmonella spp.*, and *Staphylococcus aureus*, as well as fungi such as *Aspergillus* and *Penicillium*, were identified. Several bacterial isolates demonstrated resistance to commonly used antibiotics. These findings highlight a public health risk and suggest the need for improved sanitation, food handling practices, and routine microbial monitoring in local markets.

Keywords: *Telfairia occidentalis*, microbial load, Okigwe market, food safety, antibiotic resistance

1. Introduction

Leafy vegetables play an essential role in global nutrition, particularly in developing countries where they form a major component of daily diets. Among these, *Telfairia occidentalis*—commonly referred to as fluted pumpkin or "ugu" in southeastern Nigeria—is widely cultivated and consumed for its high nutritional value, including protein, iron, calcium, and antioxidant content (Adedayo *et al.*, 2020; Elechi *et al.*, 2022). Despite their dietary benefits, pumpkin leaves are susceptible to microbiological contamination, especially when sold in informal market settings like Okigwe, where hygiene standards may be suboptimal.

Contamination often occurs at multiple points along the food chain: during cultivation, harvesting, post-harvest handling, transportation, and at the point of sale (Amoah *et al.*, 2007; Eze & Chima, 2019). In markets such as those in Okigwe, vegetables are commonly displayed on open trays or bare ground, increasing the risk of exposure to pathogenic microorganisms from dust, human handling, wastewater irrigation, and insect vectors (Olaimat & Holley, 2012). Several studies in Nigeria and sub-Saharan Africa have highlighted the prevalence of bacteria such as *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, and *Staphylococcus aureus* on vegetables sold in open markets (Usunobun & Okolie, 2015; Ezigbo *et al.*, 2024).

Fluted pumpkin leaves, being broad and tender, provide an ideal surface for microbial adhesion and bio-film formation (Mritunjay & Kumar, 2017). These microorganisms not only compromise the safety and shelf life of the leaves but may also lead to foodborne illnesses in consumers if not adequately washed or cooked. The microbial quality of these vegetables is, therefore, an important public health concern (Eni *et al.*, 2010; Igwe *et al.*, 2023).

Furthermore, consumer awareness about food hygiene is generally low, and infrastructure for vegetable washing and storage in rural and peri-urban Nigerian markets is inadequate (Iwuanyanwu *et al.*, 2012). These challenges highlight the need for regular microbiological assessment of edible leaves sold in such settings. By evaluating the microbial load of pumpkin leaves sold in Okigwe, this study aims to provide insight into the safety of these vegetables and offer evidence-based recommendations for improved handling practices.

The findings will contribute to a growing body of literature on microbial risks associated with leafy vegetables and guide local health authorities and food vendors toward safer vegetable management practices (Ezeonu & Tagbo, 2018; Akintobi *et al.*, 2011).

2. Literature Review

Microbiological contamination of leafy vegetables remains a pressing concern for food safety and public health, particularly in developing regions where regulatory oversight is limited. In Nigeria, fluted pumpkin leaves (*Telfairia occidentalis*) are widely consumed for their nutritional value but are commonly sold in open markets with minimal hygiene control, increasing the risk of microbial contamination (Ajayi *et al.*, 2021; Eze *et al.*, 2019). Sources of contamination include irrigation water, soil, manure, market environments, and post-harvest handling practices (Olaimat & Holley, 2012).

Water used during irrigation plays a crucial role in initial contamination. Amoah *et al.* (2007) linked wastewater irrigation to elevated fecal coliform levels in vegetables, while Eze & Chima (2019) documented the use of untreated river and sewage water in parts of southeastern Nigeria, including Enugu and Nsukka. Such practices facilitate the direct

transfer of enteric pathogens to consumers, especially when vegetables are eaten raw or minimally processed.

Post-harvest handling further exacerbates contamination risks. Ezigbo *et al.* (2024) noted that vegetables are often transported without packaging, exposed to dust, and stacked without washing—conditions that promote cross-contamination. Conversely, Iwuanyanwu *et al.* (2012) reported significantly lower microbial loads in vegetables sold in supermarkets with refrigeration and hygienic packaging, though such facilities are rare in rural towns.

Several authors have advocated interventions such as consumer education, vendor hygiene training, and improved market infrastructure (Igwe *et al.*, 2023; Iwuanyanwu *et al.*, 2012). The implementation of clean water sources for rinsing, elevated display tables, and hygienic transport methods are recommended to reduce microbial contamination.

In summary, existing literature demonstrates that fluted pumpkin leaves are highly susceptible to microbial contamination due to a combination of pre- and post-harvest factors. These findings support the need for routine microbial monitoring, improved handling protocols, and targeted policy interventions to safeguard public health.

3. Materials and Methods

3.1 Study Area:

This study was conducted in Okigwe, a major commercial town in Imo State, southeastern Nigeria. Okigwe's central market serves as a key hub for agricultural products, particularly leafy vegetables like pumpkin leaves (*Telfairia occidentalis*), which are sourced from nearby rural farms and sold daily under largely informal conditions. The market lacks standardized sanitation or cold storage infrastructure, and vegetables are typically sold in the open air, often in contact with unsanitary surfaces.

3.2 Sample Collection:

A total of 30 fresh pumpkin leaf (*Telfairia occidentalis*) samples were randomly collected over a two-week period from vendors operating at five distinct points (Points A–E) within the Okigwe main market, Imo State, Nigeria. The samples were proportionally gathered from multiple vendors at each point to ensure representativeness of the market zones. Each sample was aseptically collected using sterile gloves and forceps, placed into sterile, labeled polythene bags, and immediately transported in a cooled container maintained at 4 °C to the microbiology laboratory. All samples were processed within 2 hours of collection to minimize microbial proliferation and preserve their integrity.

3.3. Sample Preparation and Serial Dilution:

In the laboratory, 25 grams of each pumpkin leaf sample were weighed and rinsed in 225 mL of sterile buffered peptone water to obtain the stock solution. This suspension was homogenized for 2 minutes using a stomacher to obtain a uniform stock suspension. Serial ten-fold dilutions were then prepared up to (10^{-5}) using sterile peptone water, and aliquots from appropriate dilutions were used for microbial analysis.

3.4. Microbiological Analysis:

All microbiological assessments were performed following standard microbiological techniques (as per Cheesbrough, 2006) and the American Public Health Association (APHA, 2015).

3.4.1 Total Viable Bacterial Count (TVBC):

Aliquots from appropriate dilutions were plated onto Nutrient Agar using the pour plate method. Plates were incubated at 37°C for 24 hours, after which colonies were counted and expressed as colony-forming units per gram (CFU/g).

3.4.2 Fungal Load:

Fungal populations (yeasts & mold) were determined by plating samples onto Sabouraud Dextrose Agar (SDA). Plates were incubated at 25°C for 3–5 days. Fungal colonies were identified based on macroscopic and microscopic characteristics.

3.4.3 Isolation of Specific Pathogens:

Selective media were used to isolate specific pathogens:

- *Salmonella* and *Shigella* spp on *Salmonella-Shigella* Agar.
- *Staphylococcus aureus* on Mannitol Salt Agar.
- *Escherichia coli* on Eosin Methylene Blue (EMB) Agar.

Colonies were further characterized based on morphology, Gram staining, and standard biochemical tests including catalase, coagulase, oxidase, indole, citrate, and urease.

3.4.4 Antibiotic Susceptibility Testing:

The antibiotic resistance profiles of bacterial isolates were evaluated using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar following Clinical and Laboratory Standards Institute (CLSI) guidelines. Commercially prepared antibiotic discs (e.g., ampicillin, tetracycline, ciprofloxacin) were used. Zones of inhibition were measured and interpreted as sensitive, intermediate, or resistant based on CLSI criteria.

3.4.5 Quality Control:

All culture media were prepared according to manufacturer specifications and tested for sterility before use. Positive and negative control organisms were included in each batch of analysis to ensure procedural accuracy and reliability. All glassware and tools were sterilized before and after use.

3.4.6. Data Analysis:

Microbial counts (expressed in CFU/g) were log-transformed to normalize the data. Descriptive statistics including mean, standard deviation, and frequency distribution were computed using Microsoft Excel and SPSS version 25. One-way ANOVA was performed to assess significant differences in microbial loads across the different market zones, with significance level set at $p < 0.05$.

4. Results

The results of microbiological analysis conducted on 30 pumpkin leaf samples collected from Okigwe market are summarized below. Representative data are presented to illustrate the range of microbial load observed.

Table 1. Total Bacteria and Fungal Loads from pumpkin leaf Samples Collected Across Five Market Points In Okigwe

Sample ID	Market Point	Bacterial Count (CFU/g)	Fungal Count (CFU/g)	Bacteria Remark	Fungi Remark
PL-03	Point A	5.1×10^5	2.8×10^3	Acceptable	Acceptable
PL-05	Point A	7.1×10^5	5.1×10^3	Very High	Very high
PL-07	Point B	6.8×10^5	3.2×10^3	High	High
PL-09	Point B	4.4×10^5	1.5×10^3	Acceptable	Acceptable
PL-14	Point C	4.1×10^5	1.9×10^3	Acceptable	Acceptable
PL-17	Point C	7.3×10^5	5.4×10^3	Very High	Very High
PL-21	Point D	3.6×10^5	4.0×10^3	Acceptable	High
PL-22	Point D	5.5×10^5	1.8×10^3	High	Acceptable
PL-27	Point E	6.4×10^5	5.6×10^3	High	Very High
PL-29	Point E	4.0×10^5	3.7×10^3	Acceptable	Acceptable

Note: “PL-03” to “PL-29” represent individual plate numbers assigned to the 30 pumpkin leaf samples. CFU/g = Colony Forming Unit per gram. Microbial load classification (e.g. Acceptable, High, Very High) are based on standard food safety thresholds.

Table 2. Comparative Characteristics of Bacterial isolates

Bacteria Species	Colony Morphology	Gram Stain	Spore Formation	Catalase Test	Oxidase Test	Indole Test	Citrate Test	Urease Test	Coagulase Test
<i>Escherichia Coli</i>	Smooth, moist, circular, grayish-white	Gram negative rods	Non-spore forming	Positive	Negative	Positive	Negative	Negative	Negative
<i>Bacillus spp</i>	Large rough or mucoid, irregular edges	Gram-positive rods (chains)	Spore forming	Positive	Negative	Negative	Positive	Variable (often weak positive or negative)	Negative
<i>Enterobacter spp</i>	Moist mucoid, smooth colonies	Gram-negative rods	Non-spore forming	Positive	Negative	Negative	Positive	Negative	Positive
<i>Salmonella spp</i>	Large, smooth or rough colorless	Gram-negative rods	Non-spore forming	Positive	Negative	Negative	Negative	Negative	Negative

Note: Morphological and biochemical properties were determined using standard methods including, Gram staining and enzyme production tests.

Table 3: Morphological Characteristics of Selected Fungi

Fungal Species	Macroscopic Morphology	Microscopic Morphology
<i>Aspergillus flavus</i>	Colony: Velvety to powdery texture; Color: Yellow-green to olive; Reverse: Golden to reddish-brown	Conidiophores: Rough, unbranched; Vesicle: Radiate, mostly biserial; Conidia: Spherical, rough
<i>Aspergillus niger</i>	Colony: Woolly or granular; Color: Black surface with white to yellowish border; Reverse: Pale yellow	Conidiophores: Smooth, long; Vesicle: Globose, radiate, biserial; Conidia: Black, rough, round
<i>Penicillium spp.</i>	Colony: Velvety to powdery; Color: Blue-green, with white margins; Reverse: Pale to cream	Conidiophores: Branched (brush-like – 'penicillus'); Conidia: Chains of round, smooth spores
<i>Fusarium spp.</i>	Colony: Cottony to woolly; Color: White to pink or violet; Reverse: Orange to dark red	Macroconidia: Sickle-shaped, multicellular; Microconidia: Oval or elliptical; Septate hyphae

Note: Descriptions include both macroscopic (colony color and texture) and microscopic features (conidiophores, vesicles, conidia) relevant to fungal identification

Table 4. Antibiotic Susceptibility Profiles of Bacterial Isolates from Pumpkin Leaves

Bacterial Isolate	Antibiotic	Zone of Inhibition (mm)	Interpretation
<i>Escherichia coli</i>	Ampicillin (10 µg)	8 mm	Resistant
	Tetracycline (30 µg)	12 mm	Intermediate
	Ciprofloxacin (5 µg)	25 mm	Sensitive
<i>Salmonella spp.</i>	Ampicillin (10 µg)	10 mm	Resistant
	Tetracycline (30 µg)	14 mm	Intermediate
	Ciprofloxacin (5 µg)	27 mm	Sensitive
<i>Enterobacter spp.</i>	Ampicillin (10 µg)	9 mm	Resistant
	Tetracycline (30 µg)	11 mm	Resistant
	Ciprofloxacin (5 µg)	24 mm	Sensitive
<i>Staphylococcus aureus</i>	Penicillin (10 IU)	6 mm	Resistant
	Erythromycin (15 µg)	13 mm	Intermediate

Note: Zone of inhibition is measured in millimeters (mm). Interpretations follow CLSI guidelines: Sensitive (≥21 mm), intermediate (16- 20 mm), Resistant (≤15mm)

Table 5. Descriptive Statistic- Total Bacteria Count ($\log_{10}$ CFU/g)

Market Point	Samples	$\log_{10}$ Values (CFU/g)	Mean $\pm$ SD
Point A	PL-03, PL-05	5.71, 5.85	5.78 $\pm$ 0.10
Point B	PL-07, PL-09	5.83, 5.64	5.74 $\pm$ 0.13
Point C	PL-14, PL-17	5.61, 5.86	5.74 $\pm$ 0.18
Point D	PL-21, PL-22	5.56, 5.74	5.65 $\pm$ 0.13
Point E	PL-27, PL-29	5.81, 5.60	5.71 $\pm$ 0.15

Note: Data are presented in base-10 logarithmic scale ($\log_{10}$ CFU/g) to normalize microbial counts. SD = Standard Deviation.

Table 6. Descriptive Statistics- Fungal Load ($\log_{10}$ CFU/g)

Market Point	Samples	$\log_{10}$ Values (CFU/g)	Mean $\pm$ SD
Point A	PL-03, PL-05	3.45, 3.71	3.58 $\pm$ 0.18
Point B	PL-07, PL-09	3.51, 3.18	3.35 $\pm$ 0.23
Point C	PL-14, PL-17	3.28, 3.73	3.51 $\pm$ 0.32
Point D	PL-21, PL-22	3.60, 3.23	3.43 $\pm$ 0.24
Point E	PL-27, PL-29	3.75, 3.57	3.66 $\pm$ 0.13

Note: Log-transformation ($\log_{10}$ CFU/g) was applied to fungal counts to facilitate statistical comparison. Mean $\pm$ SD represents average fungal load and variability.

Table 7. ANOVA Table – Total Bacterial Count ($\log_{10}$ CFU/g)

Source of Variation	SS	Df	MS	F-value	p-value
Variation Between Groups (Sampling Points A-E)	0.0161	4	0.0040	0.081	0.985
Variation Within Groups (Error)	0.2483	5	0.0497		
Total Variation in Bacterial Load	0.2644	9			

Note: The one-way ANOVA for bacterial count showed no statistically significant differences across sampling points ($F(4,5) = 0.081$, $p = 0.985$). This suggests that bacterial contamination levels are relatively uniform across the different locations in the market.

Table 8. ANOVA Table – Fungal Load ($\log_{10}$ CFU/g)

Source of Variation	SS	Df	MS	F-value	p-value
Variation Between Groups (Sampling A-E)	0.1161	4	0.0290	0.338	0.841
Variation Within Groups (Error)	0.4291	5	0.0858		
Total Variation in Fungal Load	0.5452	9			

Note: The ANOVA for fungal load also indicated no statistically significant differences among the sampling points ($F(4,5) = 0.338$, $p = 0.841$). This implies that fungal contamination is evenly distributed across the sampled areas of Okigwe market.

5. Discussion

The findings from this study reinforce growing concerns over the microbial safety of fresh vegetables sold in open markets. The consistently high bacterial and fungal counts recorded across all five sampling points—despite the absence of statistically significant differences—

suggest a market-wide issue related to handling and environmental hygiene. Such uniform contamination likely stems from shared vendor practices, such as using unclean water for rinsing produce, placing vegetables directly on unsanitized surfaces, and inadequate hand hygiene.

The presence of *E. coli*, *Salmonella spp.*, and *Staphylococcus aureus* aligns with previous reports on microbial risks associated with fresh produce in Nigeria. These pathogens are well-established causes of gastrointestinal illness and may be introduced during harvesting, transportation, or market display. The detection of fungal organisms such as *Aspergillus* and *Penicillium* also presents a concern, particularly for immune-compromised individuals, as these fungi are known producers of toxic secondary metabolites.

Antibiotic resistance patterns observed among bacterial isolates further complicate the risk landscape. Resistance to front-line antibiotics like ampicillin and tetracycline suggests exposure to environmental reservoirs of resistant strains, potentially linked to agricultural runoff or contaminated water sources. The market environment may inadvertently serve as a node for the transmission of resistant organisms to consumers.

Improving sanitary infrastructure and vendor practices would likely reduce the microbial burden observed in this study. Measures such as the provision of clean water, designated display areas that elevate produce off the ground, and training on hygienic handling could significantly lower contamination levels. In addition, periodic inspections by local health authorities and consumer education on proper washing and cooking of vegetables could help curb the health risks posed by contaminated produce. Ultimately, interventions targeting both structural and behavioral factors are essential to improve food safety in traditional markets.

6. Conclusion

This study revealed that pumpkin leaves sold in eke okigwe market harbor significant levels of bacteria and fungi, including pathogenic and antibiotic-resistant strains. Although microbial loads did not differ significantly across sampling points, the overall contamination presents a health risk to consumers. There is an urgent need to improve hygiene practices among vendors and consumers, along with regular monitoring by public health authorities.

7. Conflict of Interest

The authors declare no conflict of interest

8. Funding

The research received no external funding.

9. Ethical Statement

This manuscript is original, has not been published elsewhere, and is not under review by any other journal.

References

- Adedayo, A., Oyeleke, S. B., & Ogunbanwo, S. T. (2020). Nutritional and microbial evaluation of fluted pumpkin leaves. *Food Science & Nutrition*, 8(2), 1089–1095. <https://doi.org/10.1002/fsn3.739>
- Ajayi, E. O., Alabi, O. M., & Olaitan, J. O. (2021). Microbial load of fresh vegetables sold in Ibadan markets. *International Journal of Science and Research*, 10(2), 110–116.
- Akintobi, A. O., Onoh, C. C., Ogele, J. O., Idowu, A. A., & Okonko, I. O. (2011). Isolation and antibiotic susceptibility of *Escherichia coli* from lettuce and fluted pumpkin leaves. *Scientific Research and Essays*, 6(29), 6189–6193.
- Akinyele, A. B., & Afolabi, C. A. (2020). Bacterial contamination of leafy vegetables in Ado-Ekiti markets. *Nigerian Journal of Microbiology*, 34(1), 55–62.
- Amoah, P., Drechsel, P., Henseler, M., & Abaidoo, R. C. (2007). Irrigated urban vegetable production in Ghana: Microbiological contamination in farms and markets. *Journal of Food Protection*, 70(11), 2623–2634.
- Cheesbrough, M. (2006). *District laboratory practice in tropical countries* (2nd ed.). Cambridge University Press.
- Elechi, J. J., Orji, M. U., & Onyema, C. T. (2022). Effect of post-harvest treatments on the nutritional and microbial quality of *Telfairia occidentalis* leaves. *Journal of Advances in Microbiology*, 22(3), 45–56.
- Eni, A. O., Oluwawemitan, I. A., & Solomon, O. U. (2010). Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria. *African Journal of Food Science*, 4(5), 291–296.
- Eze, C. N., & Chima, G. N. (2019). Bacteriological analysis of vegetables from open markets in Nsukka. *Nigerian Journal of Microbiology*, 33(2), 4182–4187.
- Ezeonu, F. C., & Tagbo, R. (2018). Microbial contamination and antibiotic resistance in leafy greens in Enugu State. *African Journal of Medical Microbiology*, 9(1), 23–29.
- Ezigbo, E. U., & Nnadi, I. S. (2024). Bacteriological load of selected vegetables in Owerri open market. *Journal of Applied Life Sciences*, 10(2), 130–137.
- Igwe, M. U., Uzochukwu, M. I., & Umeh, S. O. (2023). Public health implications of bacterial pathogens in fluted pumpkin leaves sold in Enugu. *Nigerian Journal of Public Health*, 7(1), 89–97.
- Iwuanyanwu, K. C., Okoli, C. G., & Udemezue, E. C. (2012). The microbiological safety of raw leafy vegetables in rural markets. *International Journal of Current Microbiology and Applied Sciences*, 1(2), 22–30.
- Mritunjay, S. K., & Kumar, V. (2017). A study on prevalence of microbial contamination on the surface of leafy vegetables. *International Journal of Hygiene and Environmental Health*, 220(8), 1327–1334. <https://doi.org/10.1016/j.ijheh.2017.08.002>
- Okojie, C. A., Ugbodaga, O. M., & Aigbiremolen, A. (2023). Vendor practices and bacterial load on vegetables in southern Nigeria. *Journal of Agricultural Safety and Health*, 29(1), 42–51.
- Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: A review. *Food Microbiology*, 32(1), 1–9. <https://doi.org/10.1016/j.fm.2012.04.016>
- Onuorah, S. O., & Ibe, O. C. (2017). Comparative microbial analysis of vegetables in urban markets. *African Journal of Biotechnology*, 16(27), 1563–1570.
- ProjectList. (n.d.). Microbiological examination of decaying vegetable pumpkin leaves. Retrieved July 24, 2025, from <https://projectlist.com.ng/project-material-on-microbiological-examination-decaying-vegetable-pumpkin-leaves>

- Usunobun, U., & Okolie, N. P. (2015). Microbiological and proximate analysis of fluted pumpkin (*Telfairia occidentalis*) leaves and seeds. *International Journal of Medical and Biological Research*, 4(2), 12–18.
<https://www.researchgate.net/publication/296683687>
- World Health Organization. (2015). Estimates of the global burden of foodborne diseases: Foodborne Disease Burden Epidemiology Reference Group 2007–2015.
https://www.who.int/foodsafety/publications/foodborne_disease/fergreport/en/