



## Isolation and Identification of Bacteria Present on *Telfairia occidentalis* Sold at Some Selected Markets in Abuja Municipal Area Council, Nigeria

\*<sup>1</sup>Ayoade, B.R., <sup>1</sup>Durugbo, A.C., <sup>2</sup>Dada, J.O. and <sup>2</sup>Eze, C.C.

<sup>1</sup>Department of Microbiology, Faculty of Basic Science, Ave Maria University, Piyanko, Nasarawa State, Nigeria

<sup>1</sup>Department of Microbiology, Faculty of Basic Science, Ave Maria University, Piyanko, Nasarawa State, Nigeria

<sup>2</sup>Department of Biotechnology, Faculty of Basic Science, Ave Maria University, Piyanko, Nasarawa State, Nigeria

<sup>2</sup>Department of Microbiology, Faculty of Basic Science, Ave Maria University, Piyanko, Nasarawa State, Nigeria

\*Corresponding Author's Email: [ayoadebiiodun6@gmail.com](mailto:ayoadebiiodun6@gmail.com)

Received on: April, 2025

Revised and Accepted on: May, 2025

Published on: July, 2025

### ABSTRACT

*Telfairia occidentalis*, commonly known as fluted pumpkin or 'Ugu' is a widely consumed leafy vegetable in Nigeria due to its high nutritional value. However, the processes involved in its planting, harvesting, and marketing particularly in open markets are often unhygienic, exposing the vegetable to potential bacterial contamination. Thirty (30) samples were aseptically collected randomly in sterile Ziploc polythene bags in batches of ten (10) from three (3) different markets namely Nyanya (Ny), Kurudu (Ku) and Karshi (Ka) in Abuja Municipal Area Council, F.C.T, Nigeria. *Staphylococcus*, *Erwinia*, *Pseudomonas*, *Enterobacter* and *Klebsiella* species were isolated and identified using Morphological, Cultural and Biochemical assessment. Ten (10) isolates each constituting of *Staphylococcus* species were identified from three (3) markets. The number of *Klebsiella* specie isolated were 6, 5 and 5 in Ny, Ku, and Ka respectively. One (1) isolate each, of *Pseudomonas* and *Enterobacter* species were identified from Ny, Ku, and Ka. Total Mean Viable Count (TVC) and Total Mean Coliform Count (TCC) were  $1.33 \times 10^6$  CFU/ml and  $1.12 \times 10^6$  CFU/ml for Ny and Ka respectively. The lowest mean count TVC and TCC were recorded in Ka. The observed differences in mean bacterial counts were not statistically significant at the 5% level ( $F = 2.83$ ,  $p = 0.0805$ ). Ny had the highest microbial load compared to Ku and Ka. This could be as a result of improper handling during harvesting and poor sanitary measures by food vendors. Hence, there should be proper public enlightenment on the handling and consumption of vegetables. **Keywords:** *Telfairia occidentalis*, Fluted pumpkin, leafy vegetable, bacterial contamination, TVC, TCC

### 1.0 INTRODUCTION

Over the years, vegetables have assumed an essential part of the dietary plan in every society. It reflects a vital food plan and the cornerstone of healthy dietary recommendations (Wallace *et al.*, 2020). They are the vital constituents of the human eating regimen providing essential dietary components preserving prime health. Among diverse varieties, leafy vegetables stand out at a distinct position due to their nutrient balance and culinary diversity. (Gupta *et al.*, 2022). As constantly recommended by the World Health Organization (WHO), the consumption of adequate levels of fruit and vegetables (F&V) represents one of the fundamental elements of a healthy diet. (WHO, 2023). Vegetables are widely produced and consumed in Nigeria. Indigenous leafy vegetables such as fluted pumpkin (*Telfairia occidentalis*), scent leaf (*Ocimum gratissimum*), and bitter leaf (*Vernonia amygdalina*) are commonly available in local markets and provide both nutritional value and income opportunities for many households (Oyewole & Olugbodi, 2025).

The fluted pumpkin (Figure 1) is an indigenous vine and a member of the cucurbit family. While the fruit isn't edible, the leaves are a famous soup green, and the

seeds are high in protein. *T. occidentalis* seeds and leaves are consumed because they are healthy sources of lipids, vitamins, fiber, and minerals such as iron, potassium, phosphorus, and mineral salt (Obembe *et al.*, 2021, Kaur *et al.*, 2020). It is now gradually grown in the northern part of Nigeria.

Fluted pumpkins (*Telfairia occidentalis*) grow in poor soil and are fairly drought resistant, making them a good choice for any Nigerian. The intricacy associated with the supply of fresh agricultural produce in the market places might significantly account for the contamination of vegetables. Collecting, processing, and delivering the products describe critical steps in the distribution of vegetables. Indeed, the disparities in these processes may lead to too much unintentional contamination of the vegetables sold in many marketplaces. (Okafor, 2022). Foodborne pathogens can be introduced from the environment either directly or indirectly during harvesting, packing (field or packing house), and transportation. Various unit operations should be considered within the context of harvest and packing of fresh fruits and vegetables, all of which can significantly change the likelihood of microbiological hazards being introduced. Crops can be

harvested by hand or mechanically as a mono- or polyculture. (FAO and WHO, 2023).



**Figure 1:** *Telfairia occidentalis* (Imosemi *et al.*, 2020)

## 2.0 MATERIALS AND METHODS

### 2.1 Sample collection

The *Telfairia occidentalis* (Fluted pumpkin or Ugu) were randomly purchased from three different markets in Abuja Municipal Area Council (Old Karshi, Kurudu and Nyanya) Abuja, Nigeria. These vegetables were randomly collected from different vendors in batches of 10 (ten) from each market. A total of 30 samples were examined to obtain their qualitative estimation of bacterial contamination. Each vegetable was stored in a separate sterile ziploc polythene bag to prevent cross contamination and transported to the Microbiology Laboratory of Ave Maria University, Piyanko Nasarawa for further analysis (Muhammad *et al.*, 2021).

### 2.2 Preparation of media

All media used (Nutrient, MacConkey, Manitol Salt, Eosine Methylene Blue Agar) were prepared according to the manufacturer's instruction. They were sterilized by autoclaving at 121°C for 15 minutes and allowed to cool to about 45–50°C, then dispensed aseptically into sterile Petri dishes at 20ml per plate (Takehira *et al.*, 2025).

### 2.3 Preparation of Samples

Twenty-five (25) g of the vegetables were soaked in 225 ml of normal saline for 10 minutes to get the inoculum for each vegetable respectively. Nine (9) ml of peptone water was introduced into each test tube using sterile pipette. Serial dilution was performed using separate sterile pipette i.e. 1ml from the inoculum was introduced into the first test tube ( $10^{-1}$ ). It was then mixed thoroughly and 1ml was aseptically introduced into the second test tube ( $10^{-2}$ ). The same procedure was done for the rest of the test tubes to seven-fold dilution. Exactly 1ml of each of the diluent was aseptically introduced into corresponding labelled sterile Petri dishes. Already prepared Nutrient and MacConkey agar

were poured slowly into the petri dishes containing the inoculum then slightly swirled for uniform distribution of the medium. The plates were then incubated at 37°C for 24 hours (Muhammad *et al.*, 2021).

### 2.4 Bacterial count

Emerging colonies on the plates were counted as Colony Forming Units per millilitre (CFU/ml). Distinct colonies were sub-cultured from Nutrient Agar to Manitol Salt Agar and MacConkey Agar to Eosine Methylene Blue respectively, until pure cultures were obtained (Muhammad *et al.*, 2021).

### 2.5 Gram staining

Bacterial smears were prepared on a grease-free slide by placing a drop of sterile distilled water and emulsifying a colony from representative agar plate using a cooled, sterilized wire loop (Panwar *et al.*, 2023).

### 2.6 Biochemical Test for the identification of bacterial isolates

#### 2.6.1 Catalase test

**With the aid of a sterile glass rod, a portion of the bacterial colonies were transferred onto clean slides. Two (2) drops of hydrogen peroxide were placed on the organism and left for a few minutes. Production of gas bubbles indicates a positive reaction (Kumar *et al.*, 2021)**

#### 2.6.2 Indole test

**The peptone water media were inoculated and incubated for 48 hours at 37°C. The tubes were then allowed to stay for an additional 48 hours in the incubator for the accumulation of indole. After this period, 0.5 ml of kovac's reagent was added separately to each test tube and swirled gently. The appearance of a red color in the alcohol layer indicated a positive reaction (Muthusamy *et al.*, 2023).**

#### 2.6.3 Methyl red test

With the aid of a sterile wire loop, the MRVP media were slightly inoculated with the test isolate and incubated at room temperature for 2 days. After the incubation period, 5 drops of methyl red reagent were added to each culture tube. Production of red color immediately indicates a positive reaction, while yellow indicates a negative result (Aryal, 2022).

#### 2.6.4 Voges-proskauer test

Sterile media were inoculated and incubated at 37°C for 48 hours. One (1) ml of 40% KOH and 3 ml of 5% alpha-naphthol in absolute alcohol (ethanol) were added. A positive test is shown by the development of pink color in 2-5 minutes, which became crimson in 30 minutes (Aryal, 2022).

### 2.6.5 Citrate utilization test

The citrate utilization test determines if an organism can use citrate as its sole carbon source, typically identifying Enterobacteriaceae such as *Klebsiella pneumoniae* (positive) and *Escherichia coli* (negative). Simmon's citrate agar contains sodium citrate, ammonium salts as a nitrogen source, and bromothymol blue as a pH indicator green at pH 6.9 and turning blue above 7.6. After inoculation from a pure colony onto the slanted surface of sterile agar, tubes were incubated at 35–37 °C for 24–48 hours. A blue slant indicates citrate metabolism (alkaline byproducts), while green indicates a negative result (MicrobeNotes, 2022; American Society for Microbiology, 2023).

### 2.6.6 Urease test

Using a sterile wire loop, heavy inoculums were collected and streaked onto the slant surface of the urea agar medium. The tubes were then incubated at 37 °C for 24 hours. A pink-red color in the medium confirmed urease activity, whereas no color change (remaining yellow or orange) indicated a negative result (Forbes *et al.*, 2022).

### 2.6.7 Triple sugar iron agar test

TSI agar is a differential medium that tests a bacterium's ability to ferment three sugars—glucose (0.1%), lactose (1%), and sucrose (1%)—alongside detecting hydrogen sulfide (H<sub>2</sub>S) production via iron salts and phenol red as a pH indicator. After inoculating by stabbing the butt and streaking the slant, tubes were incubated aerobically at **35–37 °C for 18–24 hours**. A yellow slant and butt (**A/A**) indicates fermentation of lactose and/or sucrose in addition to glucose; a red slant with a yellow butt (**K/A**) signals glucose fermentation only; red slant and butt (**K/K**) indicates no fermentation. Gas production is evidenced by bubbles or fissures, and blackening in the butt signifies H<sub>2</sub>S production (Food Microbiology Academy, 2025).

### 2.6.8 Coagulase test

The coagulase test is a biochemical test used to detect the presence of the enzyme coagulase, which converts plasma fibrinogen to fibrin, resulting in visible clotting. This test helps differentiate *Staphylococcus aureus* (coagulase-positive) from other *Staphylococcus* species. Two types of coagulase tests were used: the slide test and the tube test. For the slide test, a colony from a fresh culture was emulsified in a drop of saline on a clean glass slide, after which a drop of rabbit plasma was added. The appearance of clumping within 10 to 30 seconds indicated a positive result. For the tube test, a colony was inoculated into 0.5 mL of rabbit plasma and incubated at 37°C for 4 to 24 hours. Clot formation at any point indicated a positive result, while the absence of a clot after 24 hours was considered negative (Microbiology Info, 2023).

## 3.0 RESULTS AND DISCUSSION

### 3.1 Type and frequency of Bacteria isolated from *Telfairia occidentalis* (Ugu) collected from the three selected markets

The types and frequency occurrence of the bacteria isolated from the selected markets is presented in Table 1. *Staphylococcus*, *Klebsiella*, *Erwinia*, *Pseudomonas*, *Enterobacter*, *Bacillus* species were the organisms isolated. Vegetables constitute a large micro-biota of different bacteria species. The presence of *Staphylococcus* and *Klebsiella* species as predominant species could be attributed to improper handling during harvesting, post harvesting and poor sanitary measures by food vendors. This is in accordance the findings of Muhammad *et al* (2021) and Lenox and Nkra (2016) who also isolated different bacteria predominantly *Staphylococcus* species and *Klebsiella* species from fluted pumpkin. In contrast Okafor (2022) and Musa (2021) reported *Streptococcus* species as isolates from fluted pumpkin, bitter leaf and scent leaf.

**Table 1: Types and Frequency of Bacteria isolated from *Telfairia occidentalis* (Ugu) from the three selected markets**

Markets	Types of Organism isolated	Number of isolated organism	Percentages (%)
<b>Nyanya</b>	Staphylococcus	10	100
	Klebsiella	6	60
	Pseudomonas	1	10
	Enterobacter	1	10
	Erwinia	1	10
	Bacillus	1	10
<b>Kurudu</b>	Staphylococcus	10	100
	Klebsiella	5	50
	Pseudomonas	1	10
	Enterobacter	1	10
	Erwinia	2	20
	Bacillus	1	10
<b>Karshi</b>	Staphylococcus	10	100
	Klebsiella	5	50
	Pseudomonas	1	10
	Enterobacter	1	10
	Erwinia	2	20
	Bacillus	1	10

### 3.2 Cultural, morphological and biochemical characteristics of bacteria isolated from *Telfairia occidentalis* (Ugu) collected from the three selected markets

Isolates appeared creamy, oval and non-elevated in terms of shape, elevation and pigmentation on Nutrient Agar plates while on MacConkey Agar they appeared pinkish, oval and elevated. On microscopic examination isolates from NA plates appeared clustered cocci with purple coloration after Gram staining indicating Gram positive result. On the other hand, isolates from MAC plates appeared as straight rod with pinkish coloration after Gram staining indicating Gram negative result. On biochemical assessment, isolates tested positive for catalase and coagulase, and negative for indole in line with the result of Okafor (2022) and Musa (2021) who also reported catalase positive, coagulase positive, and indole negative results in their separate studies. Biochemical assessment is crucial for identifying specific species of microorganisms.

### 3.3 Total Mean Viable Count and Coliform Count for the three selected markets

The microbial quality of *Telfairia occidentalis* varied slightly among the three markets. Nyanya market recorded the highest mean Total Viable Count at **1.33 X 10<sup>6</sup> CFU/ml**, indicating that this location had the most bacterial presence on the samples analyzed. This

was followed closely by Kurudu, which showed a Total Viable Count of **1.23 X 10<sup>6</sup> CFU/ml**, while Karshi exhibited the lowest Total Viable Count at **0.77 X 10<sup>6</sup> CFU/ml**. When considering the Total Coliform Count, Karshi surprisingly recorded the highest mean value at **1.12 X 10<sup>6</sup> CFU/ml**. Nyanya and Kurudu recorded slightly lower TCCs at **1.10 X 10<sup>6</sup> CFU/ml** and **0.97 X 10<sup>6</sup> CFU/ml** respectively, suggesting a relatively better, albeit still concerning, microbiological status in terms of coliform contamination. Fresh vegetables are vulnerable to microbial contamination from the point of production to the point of consumption. The microbial load of 1.33 X 10<sup>6</sup> CFU/ml of the *Telfairia occidentalis* (Fluted pumpkin) that was identified in this study did not meet up with the microbial load safety record of 10<sup>3</sup> CFU/ml as directed by Food and Drug Agency. This was in line with Lenox and Nkra (2016) who reported that the microbial load of 1.45 X 10<sup>5</sup> CFU/ml of the fluted pumpkin leaves observed in their study did not meet up with the microbial load safety record of 10<sup>3</sup> CFU/ml as directed by Food and Drug Agency. On the other hand, Okafor (2022) and Musa (2021) reported 2.6 X 10<sup>4</sup> CFU/ml as microbial load *Telfairia occidentalis* (Fluted pumpkin). Vegetables particularly the leafy green vegetables currently pose the greatest concern in terms of microbiological hazards. (FAO & WHO, 2023).

**Table 2: Total Mean Viable Count and Coliform Count for the three selected markets**

Samples source	Total Viable Count (CFU/ml)	Total Coliform Count (CFU/ml)
Nyanya	1.33 X 10 <sup>6</sup>	1.12 X10 <sup>6</sup>
Kurudu	1.23 X 10 <sup>6</sup>	1.10 X10 <sup>6</sup>
Karshi	0.77 X 10 <sup>6</sup>	0.97 X10 <sup>6</sup>

**KEY**

CFU- Colony Forming Unit  
ml- Millilitre

3.4 Analysis of Data Generated from the Study

3.4.1 ANOVA Results Comparing Viable Bacterial Count across the three selected markets

The mean count observed in Karshi was 1.11 X 10<sup>6</sup> CFU/ml with a standard deviation of 0.30. Kurudu had a higher mean viable count of 1.37 X 10<sup>6</sup> CFU/ml and a slightly higher variability indicated by a standard

deviation of 0.37 X 10<sup>6</sup> CFU/ml. Nyanya showed the highest mean bacterial count at 1.48 X 10<sup>6</sup> CFU /ml with the least variability among the three, having a standard deviation of 0.26. Although the differences in the mean counts across the markets suggest a trend where Nyanya has the highest level of viable bacterial contamination followed by Kurudu and Karshi respectively, the statistical test did not yield a p-value below the conventional threshold of 0.05. This implies that the observed differences in mean bacterial counts are not statistically significant at the 5% level (F = 2.83, p = 0.0805). The R-squared value of 0.2047 indicates that approximately 20.47% of the variation in viable bacterial count can be attributed to the market location.

**Table 3: ANOVA Results Comparing Viable Bacterial Count across the three selected markets**

Outcome Measure	N	Market	Mean± SD	R-squared	f-value	p-value
Viable Count	7	Ka	1.11 x 10 <sup>6</sup> ± 0.30 x 10 <sup>6</sup>	0.2047	2.83	0.0805
	9	Ku	1.37 x 10 <sup>6</sup> ± 0.37 x 10 <sup>6</sup>			
	9	Ny	1.48 x 10 <sup>6</sup> ± 0.26 x 10 <sup>6</sup>			

p < 0.05 indicates statistical significance

Key:

ANOVA: Analysis of Variance

Ka: Karshi

Ku: Kurudu

Ny: Nyanya

R- Squared- coefficient of determination

F- Value – Fishers value

P- Value – probability value

3.4.2 ANOVA Results Comparison of Coliform Bacterial count across the three selected markets

The mean coliform count recorded in the Karshi market was approximately 1.39 CFU/ml. In Kurudu, the mean count was slightly lower at 1.23 CFU /ml, while Nyanya exhibited a mean count of 1.25 CFU /ml with a relatively smaller variability (± 0.24). Dike-Ndudim *et al.*, 2021 reported similar pattern of variability of bacterial count associated with fermented melon seed for Ogiri sold at Owerri, Imo State, Nigeria. These values suggest that all three markets had relatively high

levels of coliform contamination (Ezeakonamba *et al.*, 2025). However, the results of the ANOVA indicate that these differences in the coliform counts across the three markets are not statistically significant (F = 0.25, p = 0.7794) in accordance with Dike-Ndudim *et al.*, 2021 and Muhammed *et al.*, 2021. The R-squared value, which represents the proportion of variance in the coliform counts explained by market differences, is very low at 0.0224, suggesting that less than 3% of the variation in bacterial counts can be attributed to the market of purchase.

**Table 4: ANOVA Results Comparing Coliform Bacterial Count across the three selected markets**

Outcome Measure	N	Market	Mean± SD	R-squared	f-value	p-value
Coliform Count	7	Ka	1.39 x 10 <sup>6</sup> ± 0.71 x 10 <sup>6</sup>	0.0224	0.25	0.7794
	9	Ku	1.23 x 10 <sup>6</sup> ± 0.41 x 10 <sup>6</sup>			
	9	Ny	1.25 x 10 <sup>6</sup> ± 0.24 x 10 <sup>6</sup>			

p < 0.05 indicates statistical significance

Key:

ANOVA: Analysis of Variance

F- Value – Fishers value

P- Value – probability value

SD: Standard Deviation

R- Squared- coefficient of determination

Ny: Nyanya

**Ka: Karshi**  
**Ku: Kurudu**

#### 4.0 CONCLUSION

Vegetables are vital constituents of the human eating regimen providing essential dietary components preserving prime health. Fluted pumpkin (*Telfairia occidentalis*) also known as 'Ugu' is a widely consumed leafy vegetable in Nigeria due to its high nutritional and medicinal value. However, because it is often consumed either raw or slightly cooked, any bacterial contamination it carries can directly affect public health. The markets examined within this study indicated a very high microbial load, this may be due to uniformly poor hygiene standard across the vendors sampled in this study. When both viable and coliform counts are considered, **Nyanya appeared to maintain a consistently high microbial load**, which might reflect persistent environmental or handling-related issues. **Karshi showed the widest variability**, particularly in coliform count, pointing to inconsistent hygiene or variable environmental exposure. **Kurudu, while not the worst in either metric, presented a troubling combination of high median values for both viable and coliform counts**, suggesting it may pose the highest routine exposure risk to consumers. Therefore, Consumers are advised to thoroughly wash and carefully handle vegetables before consumption to reduce microbial contamination.

#### REFERENCES

American Society for Microbiology. (2023). *Citrate test protocol*.

Aryal, S. (2022) Methyl Red (MR) Test-Principle, Procedure and Result Interpretation <https://microbiologyinfo.com/methyl-red-mr-test-principle-procedure-and-result-interpretation/>

Aryal, S. (2022). Voges-proskauer Test-Principle, Procedure and Result Interpretation <https://microbiologyinfo.com/voges-proskauer-test-principle-procedure-and-sresult-interpretation/>

Dike-Ndudim, J.N., Ndubueze, C.W., Ezibe, J.C. & Okecukwu, E. (2021). Isolation and Identification of bacteria associated with fermentation of melon seed for Ogiri sold in Owerri, Nigeria. *Magna Scientia Advanced Research and Reviews* 03(01): 057-061

Ezeakonamba, V.M., Ezeakonamba, G.C., Ezeakonamba, C.C. (2025). Microbiological load of Pumpkin (Ugu) leaves (*Telfairia occidentalis*) sold at Eke Okigwe market, Imo State, Nigeria. *Open Access Journal of Science Research* 3(2): 59-69

FAO/WHO. (2023). Commodity-specific intervention recommendations for control of microbiological hazards in fresh fruits and vegetables. Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment

Food and Agriculture Organization of the United Nations & World Health Organization. (2023). Prevention and control of microbiological hazards

in fresh fruits and vegetables – Parts 1 & 2: General principles (Microbiological Risk

Food Microbiology Academy. (2025, February 26). Biochemistry of reactions in Triple Sugar Iron agar.

Forbes, B. A., Sahm, D. F. & Weissfeld, A. S. (2022). *Bailey & Scott's diagnostic microbiology* (15th ed.). Elsevier.

Gupta, N., Yadav, K. K., Kumar, V., Prasad, S., Cabral-Pinto, M. M. S., Jeon, B.-H., Kumar, S., Abdellattif, M. H. & Alsukaibia, A. K. D. (2022). Investigation of heavy metal accumulation in vegetables and health risk to humans from their consumption. *Frontiers in Environmental Science*, 10, Article 791052.

Imosemi, O. E., Etim, E. E., & Inyang, E. J. (2020). Morphology and distribution of species of the family C ucurbitaceae in Akwa Ibom State, Nigeria.

Kaur, S., Panghal, A., Garg, M. K., Mann, S., Khatkar, S. K., Sharma, P., & Chhikara, N. (2020). Functional and nutraceutical properties of pumpkin: A review. *Nutrition & Food Science*, 50(2), 384–401.

Kumar, S., Choudhary, A. K., Suyal, D. C., Makarana, G., & Goel, R. (2022). Leveraging arsenic-resistant plant growth-promoting rhizobacteria for arsenic abatement in crops. *Journal of Hazardous Materials*, 425, 127965.

Lennox, J. A., & Nkra, B. O. (2016). Microbiological and proximate analysis of fluted pumpkin (*Telfairia occidentalis* [F] Hook) leaves and seeds. *International Journal of Sciences*, 5(2), 12–15.

Microbiology Society. (2025). *Bacteria: Shapes and morphology*. Microbiology Society.

Muhammad, A. S., Ani, S. A., & Nasiru, A. M. (2021). Isolation and identification of bacteria responsible for the spoilage of fluted pumpkin (*Telfairia occidentalis*) and bitter leaf (*Vernonia amygdalina*) in Sokoto Metropolis. *EPRA International Journal of Multidisciplinary Research* 7(1), 34–38.

Musa, S.A. (202). Isolation and Identification of Bacteriological Composition of some selected Vegetable leaves sold in markets. *International Journal of Advanced Research* 9(5), 521-526

Muthusamy, D., Krishnan, R., & Anbazhagan, M. (2023). A comprehensive study on biochemical characterization and antimicrobial resistance of waterborne pathogens. *Journal of Pharmaceutical Negative Results*, 14(3), 1082–1090.

MicrobeNotes. (2022). Simmons citrate agar: Composition, principle, preparation & result interpretation.

Microbe Online. (2022). Citrate utilization test: Principle, procedure, results.

MicrobiologyInfo. (2023). Coagulase test – Principle, procedure, types, interpretation, and examples.

Obembe, O.M., Ojo, D.O., & Ileke, K. D. (2021). Effect of different drying methods and storage on proximate and mineral composition of fluted

- pumpkin leaf, *Telfairia occidentalis* Hook f. *Journal of Horticulture and Postharvest Research*, 4(2), 141–150.
- Okafor, P.N. (2022). Isolation and identification of bacteriological constituents of some leafy vegetables sold in rural markets in Anambra State, Nigeria. *International Journal of Advanced Research*, 10(2), 845–851.
- Oyewole, M. F., & Olugbodi, G. O. (2025). Consumption pattern of indigenous leafy vegetables among selected households in Ibadan Zone, Nigeria. *World Nutrition*, 16(2), 47–52.
- Panwar, G., Panwar, G., & Bharti, T. (2023). *Gram staining: A fundamental technique microbiology*. Noble Science Press.
- Takehira, K., Awasthi, S. P., Hatanaka, N., Nagita, A., Hinenoya, A., & Yamasaki, S. (2025). Evaluation of a novel modified selective medium cefixime-tellurite-phosphate-xylose-rhamnose MacConkey agar for the isolation of *Escherichia albertii* from diarrheal stool specimens. *Journal of Veterinary Medical Science*, 87(3), 308–314.
- Wallace, T. C., Bailey, R. L., Blumberg, J. B., Burton-Freeman, B., Chen, C. O., Crowe-White, K. M., Drewnowski, A., Hooshmand, S., Johnson, E., Lewis, R., Murray, R., Shapses, S. A. & Wang, D. D. (2020). Fruits, vegetables, and health: A comprehensive narrative, umbrella review of the science and recommendations for enhanced public policy to improve intake. *Critical Reviews in Food Science and Nutrition*, 60(13), 2174–2211.
- World Health Organization. (2023). *Carbohydrate intake for adults and children: WHO guideline*. World Health Organization.