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Evaluation of Cytotoxicity, Minimum Inhibitory and Bactericidal Concentrations of Fluted Pumpkin Leaves Obtained from Idah Local Market, Kogi State, Nigeria

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Abstract

This study assesses the cytotoxicity bioassay and evaluates the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of fluted pumpkin (*Telfairia occidentaliss*) leaves obtained from local markets in Idah, Kogi State, Nigeria. The solvent extracts were obtained from the powdered leaf sample of fluted pumpkin using maceration extraction technique. The cytotoxicty was assessed using brine shrimp lethality bioassay. The minimum inhibitory and bactericidal concentrations were evaluated using agar diffusion methods. The results showed that the extracts showed no observable toxicity, with all the extracts' LC50 values found to be above 1000 µg/ml (n-hexane = 8265 µg/ml, ethyl acetate = 8097 µg/ml, acetone = 8311 µg/ml, ethanol = 7360 µg/ml, and methanol = 7862 µg/ml). The extracts also demonstrated promising minimum inhibitory and bactericidal concentrations against common isolates (*Staphylococcus aureus, Pseudomonas aerogunosa, Candida albican*, *Salmonella typhi, Escherichia coli,* and *Vibrio cholerae*). These findings demonstrated that fluted pumpkin leaves are both safe for consumptions and biologically active, reinforcing their traditional use and highlighting their potential as candidates for natural antimicrobial development.

Keywords: Fluted Pumpkin**,** Antioxidant properties, Antimicrobial resistance, minimum inhibitory concentration, minimum bactericidal concentration

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1. Introduction

The problem of antimicrobial resistance is still a persistent menace to the healthcare systems across the world due to the excessive use of antibiotics in both medical and agricultural industries. This trend highlights the necessity of finding new and environmentally friendly antimicrobial agents that would be sourced naturally (Allel, 2021; Diass *et al.,* 2023; Haddou *et al.,* 2023; Nikol *et al.,* 2024; Alhassan and Ahmad, 2025; Danjuma and Lawan, 2025). The World Health Organization (2023) discovered that the emergence of antimicrobial resistance in the world has caused approximately 4. 95 million deaths in 2019 and about 1.27 million of these deaths were directly caused by resistant infections. In the medical, veterinary practice and agriculture, the excessive use and misuse of antibiotics have accelerated the emergence of microorganisms resistant to various drugs. Antimicrobial resistance occurs as a result of genetic changes which can be used to prevent the effects of antibiotic treatment on microorganisms. Besides genetic resistance, other bacterial behaviours during infection, such as persistence and biofilm formation also contribute to the high chances of failure in antibiotic treatment and death of the patient (Nikol *et al.,* 2024).

The research project on economic analysis that antimicrobial resistance may cause global healthcare expenses of approximately 1 trillion US Dollars by 2050 and annual GDP of 3.4 trillion US Dollars by 2030 (WHO, 2023). These predictions indicate the urgency of exploring more novel sustainable sources of antimicrobial agents. Infection diseases remain common, especially in low- and middle-income countries in which healthcare facilities are insufficient, poverty and sanitation are poor (Emmanuel *et al.,* 2025). This has made the preservation of the shelf life of our approved antibiotics now a significant area of concern and at the same time bolstering our existing weak preclinical and clinical antimicrobial development pipelines. As another way to determine the presence of antibiotic-resistant strains in the clinic and test new antimicrobial drug or antibiotic-adjuvant candidates, both achievements largely require our ability to measure the efficacy of antimicrobial drugs against bacteria (Nikol *et al.,* 2024). Additional preclinical and clinical research on the traditionally used medicinal plants is necessary to verify their safety and biological activity (Nerdy *et al.,* 2021).

The cytotoxicity of various levels of many of the herbal plants remains unclear. One of the ways through which cancer is treated is by the use of medicinal plants. In fact, instead of altering the role played by traditional medicine, the emergence of the modern science and technology has reinforced and complemented its practices (Salmeron-Manzano *et al.,* 2020). The use of herbal remedies has increased in many Countries especially the developing countries in the past decades. This has increased their use due to the perception that their natural condition does not give them health hazards and also because these are supplemented by nutritional and financial benefits (Bouslamti *et al.,* 2023; El Hassania *et al.,* 2024; Bouammali *et al.,* 2024). Considering the adverse consequences of their use in conventional medicine reported, the investigation of plant secondary metabolites has become necessary (Kamanja *et al.,* 2018). The plant-derived compounds have played an essential role in chemotherapeutic drug development, whereas the traditional medical professionals used natural bioactive chemicals found in plants, fungi, and marine sources to treat cancer long ago (Hamidi *et al*., 2014; Kuruppu *et al*., 2019; Ouahabi *et al*., 2023). Antibiotics are antimicrobial agents that are produced by microorganisms and although they are harmless to host cells, prevent the growth of other germs or destroy them. The choice of the most appropriate antibiotic to be used in the treatment of microbiological infections depends on the knowledge of the susceptibility of the organisms to the various medications. Antibiotics are said to be bacteriostatic; they only prevent the multiplication of bacteria, but not bactericidal antibiotics that kill bacteria. Most medicines may be bacteriostatic and bactericidal based on their concentration (Chikezie and Owiama, (2017).

Antioxidant biomolecules are molecules that neutralize free radicals and protect cells against oxidative stress. They can be endogenous (synthesized by the body), such as glutathione and enzymes, or exogenous (obtained from food), such as vitamins (C and E), polyphenols, flavonoids, and carotenoids (El Guerrouj *et al*., 2023; Mrani *et al*., 2024).

Kabir and Imrana (2025) further stated that Nigerians traditionally use fluted pumpkin leaves as an antioxidant and antibacterial agents due to the presence of a wide range of phytochemicals, such as tannins, phenols, steroids, terpenoids, and alkaloids. They were also reported to be rich in vitamins B1, B2, B3, B5, B7, B12 and C (Ali *et al.,* 2024; Chijindu *et al.,* 2024; Danjuma and Lawan, 2025). Many researchers have recorded that the leaves of *T. occidentalis* have antibacterial, hematological, analgesic, anti-inflammatory, and hypoglycaemic effects. In addition, *T. occidentalis* seed oil has been demonstrated to improve testicular performance and raise sperm count, thus improving male infertility. Moreover, such antioxidants as glutathione and tocopherol are present in the fluted pumpkin and provide protection against oxidative stress in humans (Igboecheonwu *et al*., 2023; Ominakinde *et al.,* 2018). This research was conducted to evaluate the cytotoxic effect, minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of fluted pumpkin leaves obtained from local markets in Idah, Kogi State, Nigeria.

2. Material and Method

2.1 Sampling and Sample identification

This study used samples of *Telfairia occidentalis* leaves obtained from the vegetables sellers sitting along the second gate of the Federal Polytechnic Idah, Kogi State, Nigeria. The plant sample identification was carried out at Biology Department, School of Technology, Federal Polytechnic Idah.

2.2 Sample Preparation

The leaves sample of *Telfairia occidentalis* were subjected to thorough cleaning and washing under running tap water to remove debris and other impurities. The sample was then dried under shade for ten days before being grounded into powder using laboratory mortar and pestle. The powdered sample was then kept in an air and water tight containers until use (Danjuma *et al.,* 2025)

2.3 Extraction of Plant Material

To obtain crude extracts, 300 g of the powdered leaves were immersed in ethanol for 48 hours under periodic stirring, followed by filtration and solvent evaporation under ambient condition. The crude extract was further macerated with solvents of increasing polarity ((n-hexane, ethyl acetate, acetone, ethanol, and methanol solvents) to obtain their respective fractions. The fractions were air-dried and stored until further analysis began (Danjuma *et al*., 2024; Danjuma *et al.,* 2025).

2.4 Brine Shrimp Lethality Assay

In the case of the cytotoxicity experiment, brine shrimp (*Artemia salina*) larvae were used. The artificial seawater was made by dissolving 33.33 grams of sea salt in one litre of distilled water. The hatching of brine shrimp larvae was carried out using constant light conditions and aeration in the artificial seawater (prepared using sea salt and distilled water). Test concentration of 10 to 300 µg/ml of the extract was prepared by serially diluting the extract in 1% DMSO-sea water. K2Cr2O7 and artificial seawater with 1% DMSO were the positive and negative controls, respectively. Ten brine shrimp larvae were put in a test tube, and allowed to incubate in 10 milliliters of sample solutions at room temperature environment for 24 hours. Each sample and level of concentration was tested three times. The percentage mortality was determined by comparison of the results of the mean number of surviving larvae in the tests with the control (Meyer *et al.,* 1982; Sukkum *et al.,* 2025; Danjuma *et al.,* 2025).

2.4.1 Data Analysis

Descriptive statistics were produced using Microsoft Excel 2007 software, which was used to enter and handle the extraction data. Using the Finney computer program's probit approach, which counts the number of dose levels, brine shrimp for each concentration, and the percentage of mortality for each concentration and dose level, the cytotoxicity data were evaluated. Using the computer algorithm, the lethal concentration (LC50) and 95% confidence interval were determined (Kamanja *et al.,* 2018).

2.5 Minimum Inhibitory Concentration (MIC)

With minor adjustments, the MIC was calculated using the method by Mustapha *et al*. (2012). The plant extracts were prepared at 100 mg/ml in sterile distilled water and serially diluted twofold with nutrient broth to yield concentrations between 10 mg/mL to 500 mg/mL. Finally, 0.2 ml of the test organism suspension was added as inoculants. Turbidity was checked in the test tubes following an 18-hour incubation period at 37^{0}C. The lowest concentration at which no turbidity was seen was identified as the minimum inhibitory concentration (MIC) value.

2.6 Minimum Bactericidal Concentration (MBC)

Through sub-culturing to anti-microbial free agar, this was ascertained from the broth dilution obtained from the MIC tubes. Using a sterile wire loop, the contents of the test tubes containing the MIC were streaked over a bacterial-free agar plate and incubated for eighteen hours at 37^{0}C. The extract's lowest concentration at which no bacterial growth was seen was identified as the MBC (Mustapha *et al*., 2012).

Figure 1: Fluted Pumpkin Leaves

3. Result and Discussion

3.1 Cytotoxicity of the Leaf Extracts of *T. occidentalis*

Table 1 below contains the results of cytotoxicity of the fluted pumpkin leaf extracts. The result of the brine shrimp lethality assay of the n-hexane extract of Fluted Pumpkin leaves was presented in **Table 1** and the result showed that the percentage mortality increases when the concentration of the extract increases. For instance, the percent mortality values at 10 mg/ml, 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml, and 300 mg/ml were 16 %, 25 %, 27 %, 36 %, 41 %, 56 %, and 71 %, respectively. This shows that the percent mortality of 10 mg/ml to 300 mg/ml concentration increased from 16 % to 71 %. The lethal concentration (LC_{50}) of the n-hexane extract was 8265 µg/ml. The n-hexane extract is not toxic due to its LC50 being higher than 1000 µg/ml.

Table 1: Brine Shrimp Lethality assay of n-hexane extract of Fluted Pumpkin leaves

Conc. (mg/ml)	Log_{10} Conc.	% Mortality	Probit Value	LC_{50} (µg/ml)
10	1.0	16	4.01	
50	1.69	25	4.33	
100	2.0	27	4.39	
150	2.17	36	4.64	8265
200	2.30	41	4.77	
250	2.39	56	5.15	
300	2.47	71	5.56	

The result of the brine shrimp lethality assay of the ethyl acetate extract of Fluted Pumpkin leaves was presented in **table 2** and the result showed that the percentage mortality also increases when the concentration of the extract increases. For instance, the percent mortality values at 10 mg/ml, 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml, and 300 mg/ml were 21 %, 22 %, 26 %, 30 %, 43 %, 55 %, and 67 %, respectively. This shows that the percent mortality of 10 mg/ml to 300 mg/ml concentration increased from 21 % to 67 %. The probit values also increased from 4.19 to 4.44 with concentrations of the solution. The lethal concentration (LC_{50}) of the n-hexane extract was 8097 µg/ml. This LC50, being larger than 1000 µg/ml, shows that the ethyl acetate extract of fluted pumpkin leaves is not toxic.

Table 2: Brine Shrimp Lethality assay of ethyl acetate extract of Fluted Pumpkin leaves

Conc. (mg/ml)	Log_{10} Conc.	% Mortality	Probit Value	LC50 (µg/ml)
10	1.0	21	4.19	
50	1.69	22	4.23	
100	2.0	26	4.36	8097
150	2.17	30	4.59	
200	2.30	43	4.82	
250	2.39	55	5.13	
300	2.47	67	4.44	

Table 3: Brine Shrimp Lethality assay of acetone extract of Fluted Pumpkin leaves

Conc. (mg/ml)	Log_{10} Conc.	% Mortality	Probit Value	LC50 (µg/ml)
10	1.0	32	4.53	
50	1.69	33	4.56	
100	2.0	36	4.68	
150	2.17	45	4.89	8311
200	2.30	57	4.92	
250	2.39	69	5.50	
300	2.47	74	5.64	

The result of the brine shrimp lethality assay of the acetone extract of Fluted Pumpkin leaves was presented in **Table 3**. An increase in mortality percentage with increase in concentration of the plant leaf extract was observed. The percent mortality values at 10 mg/ml, 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml, and 300 mg/ml were 32 %, 33 %, 36 %, 45 %, 57 %, 69 %, and 74 %, respectively. This shows that the percent mortality of 10 mg/ml to 300 mg/ml concentration increased from 32 % to 74 %. The probit values also increased from 4.53 to 5.64 with concentrations of the solution. The lethal concentration (LC_{50}) of the n-hexane extract was 8311 µg/ml. This LC50, being larger than 1000 µg/ml, shows that the acetone extract of fluted pumpkin leaves is not toxic. This finding suggests that fluted pumpkin leaves are safe consumptions (**Figure 2**).

Table 4: Brine Shrimp Lethality assay of ethanol extract of Fluted Pumpkin leaves

Conc. (mg/ml)	Log_{10} Conc.	% Mortality	Probit Value	LC50 (µg/ml)
10	1.0	27	4.39	
50	1.69	29	4.45	
100	2.0	37	4.67	7360
150	2.17	40	4.75	
200	2.30	48	4.95	
250	2.39	53	5.08	
300	2.47	67	5.44	

The result of the cytotoxicity assay of the ethanol extract of *T. occidentalis* leaves was presented in **Table 4**. An increase in mortality percentage with increase in concentration of the plant leaf extract was observed. There was an increase in death of the species when the concentration was increased. The percent mortality values at 10 mg/ml, 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml, and 300 mg/ml were noticed to be 27 %, 29 %, 37 %, 40 %, 48 %, 53 %, and 67 %, respectively. This shows that the percent mortality of 10 mg/ml to 300 mg/ml concentration increased from 32 % to 74 %. The probit values also increased from 4.39 to 5.44 from 10 mg/ml to 300 mg/ml concentrations of the solution. The lethal concentration (LC_{50}) of the ethanol extract was 7360 µg/ml. This LC50, being larger than 1000 µg/ml, shows that the ethanol extract of fluted pumpkin leaves is not toxic.

Table 5: Brine Shrimp Lethality assay of ethanol extract of Fluted Pumpkin leaves

Conc. (mg/ml)	Log_{10} Conc.	% Mortality	Probit Value	LC50 (µg/ml)
10	1.0	19	4.12	
50	1.69	22	4.23	
100	2.0	25	4.33	
150	2.17	39	4.72	7862
200	2.30	45	4.87	
250	2.39	50	5.00	
300	2.47	66	5.41	

The result of the cytotoxicity assay of the methanol extract of *T. occidentalis* leaves was presented in **Table 5**. There was an increase in death of the species when the concentration was increased. The percent mortality values at 10 mg/ml, 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml, and 300 mg/ml were noticed to be 19 %, 22 %, 25 %, 39 %, 45 %, 50 %, and 66 %, respectively. This shows that the percent mortality of 10 mg/ml to 300 mg/ml concentration increased from 19 % to 66 %. The probit values also increased from 4.12 to 5.41 from 10 mg/ml to 300 mg/ml concentrations of the solution. The lethal concentration (LC_{50}) of the ethanol extract was 7862 µg/ml. This LC50, being larger than 1000 µg/ml, shows that the methanol extract of fluted pumpkin leaves is not toxic.

Cytotoxicity simply occurs when an agent interferes with important metabolic or physiological functions of living organism, which may result in an abnormal behavior or death. This causes abnormalities in the organ systems which may develop to abnormal behavior or death of the organism. The test is also sensitive enough to detect materials that are cytotoxic enough to kill the larvae of shrimp on exposure. According to the brine shrimp lethality model, extracts with LC50 more than 1000 u/ml are considered non-toxic, whereas being below that value indicates the possibility of toxicity (Ghosh *et al*., 2015; Sufian and Haque, 2015; Kibiti and Afolayan, 2016; Quazi *et al*., 2017; Ouahhoud *et al*., 2021; Danjuma *et al*., 2024). Over the last decade, cancer has been a significant threat to national health and the economy that causes death in people, families, healthcare systems, and economies, so it is the top killer of people all over the globe (Alzehr *et al.*, 2022). The combination of hereditary, environmental and lifestyle issues have a complex interaction that has led to the constant rise in cancer rates among the population.

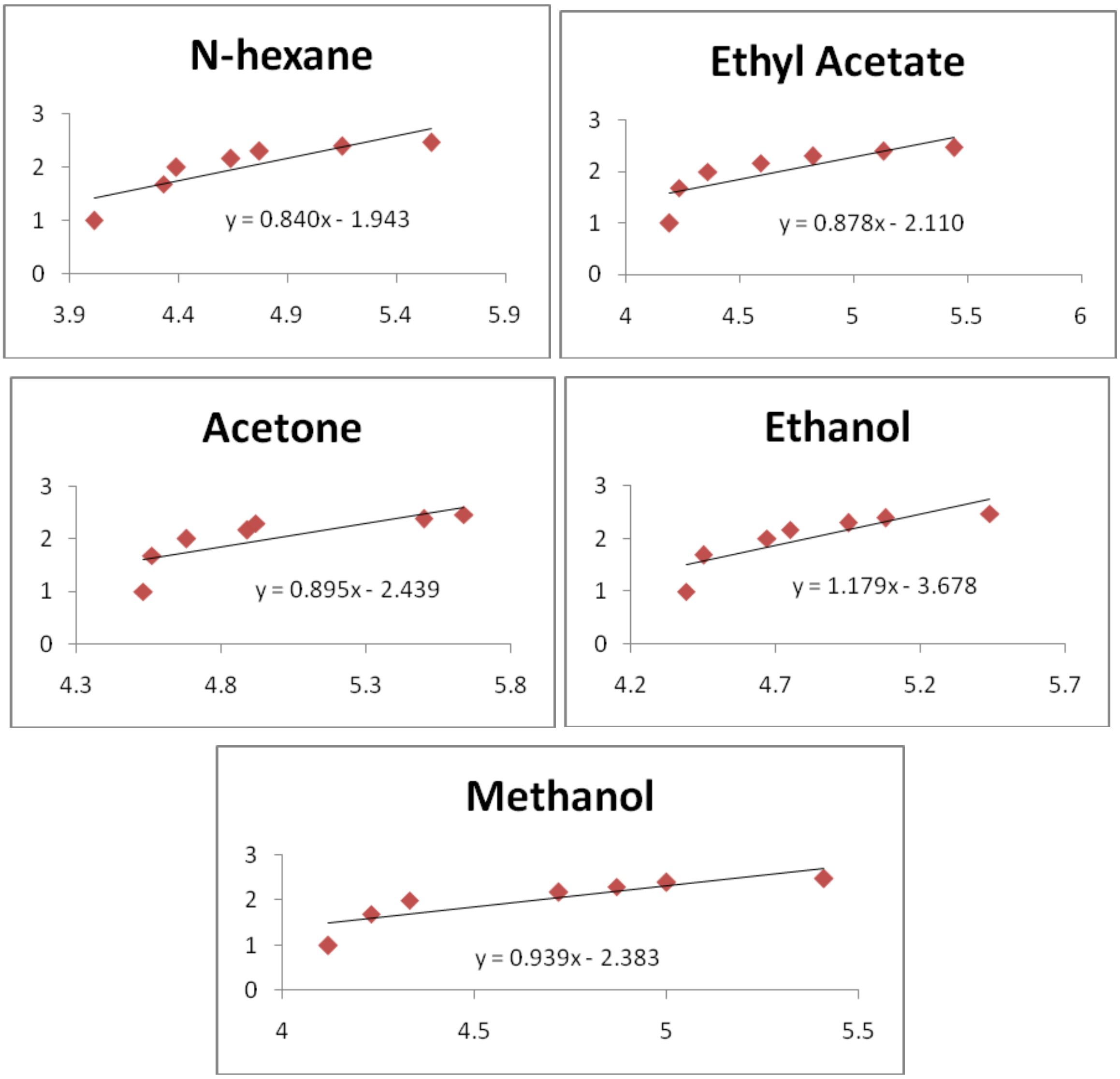

Figure 2: Regression Analysis plots of the leaf extracts of *T. occidentalis*

The traditional forms of cancer therapy are immunotherapy, radiation therapy, cytotoxic chemotherapy, or surgery that may be given alone or combined (Muralidharan *et al.,* 2023). The occurrence of flavonoids in the *T. occidentalis* extracts could be one of the reasons of the cytotoxic effects, as flavonoids may disrupt digestive or metabolic pathways in the test organism (Salmeron-Manzano *et al.,* 2020; Kabir and Imrana, 2025). Also, flavonoid compounds in plants block the taste receptors in the brine shrimp larvae to prevent its feeding thus starves to death as a result of inability to eat. The flavonoid chemicals are both in extract and pure isolate form and add to the cytotoxic effects on the brine shrimp larvae. The extract that contains more alkaloids and flavonoids is more cytotoxic and its LC50 is lower, thereby raising its anticancer activity (Salmeron-Manzano *et al.,* 2020).

3.2 Minimum Inhibitory Concentration of Fluted Pumpkin Leave Extracts

The minimum inhibitory concentration, or MIC, is the lowest concentration of an antibiotic (in µg/mL) that prevents a particular bacterial strain from growing. The result of MIC of the fluted pumpkin leaf extracts in this study is presented in **table 6.**

Table 6: Minimum Inhibitory Concentration (MIC) of the fluted pumpkin leaf extracts

Microorganisms	MIC (mg/ml)				
	n-hexane	**Ethyl acetate**	**Acetone**	**Ethanol**	**Methanol**
Staphylococcus aureus	300	100	250	100	150
Pseudomonas aerogunosa	300	250	150	NA	300
Candida albican	NA	NA	300	300	250
Salmonella typhi	100	100	300	100	150
Bacillus subtilis	NA	NA	NA	NA	NA

Key: NA = Not Reactive

Table 6 showed the outcomes of the Minimum Inhibitory Concentration (MIC) of fluted pumpkin leaf extracts. This finding revealed that *Staphylococcus aureus* had MIC of 300 mg/ml (n-hexane extract), 100 mg/ ml (ethyl acetate extract), 250 mg/ml (acetone extract), 100 mg ml (ethanol extract), and 150 mg mg/ml (methanol extract). *Pseudomonas aerogunosa* also gave MIC 300mg/ml (n-hexane), 250mg/ml (ethyl acetate), and 150mg/ml (acetone). Candida albican activity was absent in n-hexane and ethyl acetate extracts, and the activity was found at the 300 mg/ml, 300 mg/ml, and 250 mg/ml in acetone, ethanol, and methanol extracts. The *Salmonella typhi* bacterium was inhibited by n-hexane, ethyl acetate, acetone, ethanol and methanol extracts, which had a 100 mg/ml, 100 mg/ml, 300 mg/ml, 100 mg/ml and 150mg/ml concentration respectively. The presence of bioactive compounds such as tannins, phenolics, alkaloids, saponins, and flavonoids in the extracts was demonstrated by these inhibitions (Abdoulahi *et al.,* 2023; Zeleke *et al.,* 2024). Nevertheless, the *Bacillus subtilis* did not have any activity (NA) in all the extracts of fluted pumpkin leaves at all the concentrations, indicating a strong surveillance effect such as the creation of spores or layers of peptidoglycan that prevent the quality of the antimicrobial compounds of the plant. It has been reported that *Bacillus subtilis* may give rise to endspores that are dormant in metabolic terms, and that they shape them to be highly resistant to the environment and treatment factors by antimicrobials (Kamanja *et al.,* 2018).

3.3 Minimum Bactericidal Concentration of Fluted Pumpkin Leaf Extracts

The MBC data of the extracts of *Telfairia occidentalis* leaves is presented in **table 7** below. The lowest amount of an antibacterial agent needed to eradicate or kill a certain bacterium is known as the minimum bactericidal concentration. **Table 7** showed the values of Minimum Bactericidal Concentration of the fluted pumpkin leaves extracts on the target Gram-negative and Gram-positive bacterial pathogens. The activity of the extracts with the bacterial pathogens varied greatly in the microorganisms with differences in the structures of the bacteria, the mechanisms of its resistances, and the way in which they interact with the bioactive compounds of the extracts. The MBC of *Staphylococcus aureus* was 100 mg/ml (n -hexane), 100 mg/ml (ethyl acetate), 200 mg/ml (acetone), 150 mg/ml (ethanol) and 100 mg/ml (methanol), respectively. These MBC values compel the presence of bioactive phytochemicals such as saponins, tannin, alkaloids, and flavonoids. It is shown that these bioactive compounds disrupt and tear the membranes of bacteria, modify protein synthesis, and cause cell death (Salisu *et al.,* 2024; Aderele *et al*., 2020).

Table 7: Minimum Bactericidal Concentration (MBC) of fluted pumpkin leaf Extract

Microorganisms	**MBC (mg/ml)**				
	n-hexane	**Ethyl acetate**	**Acetone**	**Ethanol**	**Methanol**
Staphylococcus aureus	100	100	200	150	100
Pseudomonas aerogunosa	250	100	150	NA	200
Candida albican	NA	NA	100	300	250
Salmonella typhi	150	150	100	250	200
Escherichia coli	300	200	150	100	100
Bacillus subtilis	NA	NA	NA	NA	NA
Vibrio cholerae	100	100	150	100	100

Key: NA = Not Reactive

These fluted pumpkin leaf extracts have the possibility of responding to bacterial isolates, which validate the uses of the fluted pumpkin leaves used in the past to treat the infectious diseases such as streptococcal, pneumonia, and skin diseases. Some findings have suggested that terpenoids, which are found in several plant species including fluted pumpkin, possess pharmacological properties. It is believed that terpenoids facilitate the action of medicine since various studies have established that terpenoids have chemopreventative and chemotherapeutic properties against cancer in humans. The terpenoids, monoterpene, triterpenes, and sesquiterpenes, are useful anticancer agents applicable in the treatment of chemotherapy-resistant cancer as well as minimize the side effects of the current methods (Boncan *et al.,* 2020; Proshkina *et al.,* 2020; Yang *et al.,* 2020).

Conclusion

Fluted pumpkin leaf extracts were found to be free of toxicity and excellent antibacterial agents against diverse clinically relevant pathogens, such as *Candida albicans*, *Escherichia coli, Salmonella typhi, Pseudomonas aerogunosa* and *Vibrio cholerae*. The findings indicate that *T. occidentalis* leaf extracts have great antimicrobial potential with a low cytotoxic index, which supports their use as traditional medicines and a possible drug development.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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