MYCOTOXICOLOGY SOCIETY OF NIGERIA

In Collaboration With

AFRICA CENTRE OF EXCELLENCE FOR
MYCOTOXIN AND FOOD SAFTY
FEDERAL UNIVERSITY OF TECHNOLOGY MINNA

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IMPACT OF FUNGAL CONTAMINATION ON NUTRIENT COMPOSITION OF RICE, MAIZE, AND SORGHUM IN NIGERIA: CORRELATION ANALYSIS AND IMPLICATIONS FOR FOOD QUALITY

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ABSTRACT
This study assesses the effect of fungal contamination on the nutrient composition of staple cereals; rice (*Oryza sativa*), maize (*Zea mays*), and sorghum (*Sorghum bicolor*)—widely consumed in Nigeria. A total 23 samples of maize (7), rice (6) and sorghum (9) were analyzed for fungal colony-forming units (CFUs) via plate dilution method and nutrient composition (moisture, ash, fat, fiber, protein, and carbohydrate levels) was measured following Association of Analytical Chemists (AOAC) methods. Statistical analysis, including Spearman correlation, was applied using SPSS software. Maize showed the highest fungal contamination with a mean CFU of 4.0×10^5 CFU/g, followed by Sorghum at 3.7×10^4 CFU/g, while rice had the lowest at 2.7×10^4 CFU/g. Significant positive correlations were observed between fungal growth and moisture content in all grains, with correlation coefficients of 0.752 for rice, 0.806 for maize, and 0.550 for sorghum. Conversely, carbohydrate content showed a significant negative correlation in all samples, notably in maize (-0.860) and rice (-0.638), with (-0.462) for sorghum. Fat and fiber content showed a negative correlation in rice (-0.584) and maize (-0.488), while sorghum showed a positive correlation (0.413). Only rice showed a negative correlation for ash (-0.415) and protein (-0.136), while maize (0.354 and 0.220) and sorghum (0.258 and 0.384) showed a positive correlation. These findings underscore the nutrient losses due to fungal contamination, highlighting the necessity for enhanced post-harvest storage to mitigate contamination and preserve cereal nutritional quality.

Keywords: Fungi, Nutrient composition, Cereals.

INTRODUCTION
Cereals such as rice (*Oryza sativa*), maize (*Zea mays*), and sorghum (*Sorghum bicolor*) are essential staple foods worldwide, providing a primary source of calories and key nutrients for millions of people, especially in developing regions (Muitire *et al.*, 2021). These grains are not only foundational to food security but are also crucial in providing proteins, vitamins, and minerals necessary for balanced nutrition. However, cereal crops are highly susceptible to contamination by fungi during growth, harvest, and storage, which poses significant risks to both food safety and nutrient quality (Agriopoulou, 2021; Habschied *et al.*, 2021; Kumar *et al.*, 2021).

Fungal contamination in cereals is predominantly caused by several species such as *Aspergillus, Fusarium*, *Penicillium*, and *Alternata* species which thrive in warm and humid environments often associated with grain storage in tropical and subtropical climates (Chasna *et al.*, 2024; Cruz-Luna *et al.*, 2021). These fungi not only damage and decrease the shelf life of grains rendering them unfit for human consumption (Majumder *et al.*, 2013; Nešić *et al.*, 2021), but also produce mycotoxins, which are secondary metabolites that are toxic to humans and animals (Liu *et al.*, 2020). The damages caused by these microorganisms to cereals are estimated to be approximately between 20 to 40 % in Africa (Adégbola *et al.,* 2011). Mycotoxins, such as aflatoxins, ochratoxins, fumonisins, patulin, and Alternaria toxins, are well-known for their health risks, including neurotoxicity, carcinogenicity, and immunotoxicity. Beyond these health concerns, fungal contamination can lead to nutrient depletion in contaminated grains, as the microorganisms consume essential nutrients, impacting the grain's nutritional profile and, consequently, human health (Nishimwe *et al.*, 2020).

The impact of fungal contamination on the nutrient composition of rice, maize, and sorghum remains a crucial area of study. Understanding how fungi alter the levels of macronutrients (carbohydrates, proteins, fats) in these grains can inform agricultural practices, storage solutions, and mycotoxin management strategies. In this study, the effect of fungal

contamination on the nutrient composition of rice, maize, and sorghum was examined and highlights the potential nutritional and public health implications of consuming contaminated cereals.

MATERIALS AND METHODS

Collection of samples

The use of sample materials in this study complies with relevant institutional, national, and international guidelines and legislation.

The grains of rice (6), maize (7), and sorghum (9) were obtained with permission from farms and stores; and purchased from markets in different agroecological zones in Nigeria.

Sample preparation

250 grams of each sample was milled into a fine powder using a mixer grinder. To avoid cross-contamination, cleaning and decontamination of the equipment was performed using methanol after each milling step. To also prevent further contamination, all samples were kept in the freezer.

Fungi isolation procedure

Fungi isolation was carried out using the plate dilution method as described by (Nevalainen *et al.,* 2014). One gram of the milled sample was weighed into a sterile tube suspended in 9 mL of distilled water and shaken to make a first dilution of 10-1. Three serial dilutions were carried out for maize to obtain 10^{-1}, 10^{-2}, and 10^{-3} dilutions, while two serial dilutions were carried out for rice and sorghum to obtain 10^{-1} and 10^{-2}. One milliliter of each diluent was plated onto potato dextrose agar (PDA). The plates were incubated for 2 days at room temperature. After incubation, the growth of fungi colonies was observed and counted, the number of colonies per gram of samples was expressed in colony forming unit per gram (CFU/g).

Proximate analysis of rice, maize and sorghum

Standard methods by the Association of Analytical Chemists (AOAC, 2005) were adopted in the analysis. The system consists of the analytical determination of moisture, ash, crude fat, crude fibre, crude protein and carbohydrate.

Determination of moisture content

Each of the samples were weighed, dried in a hot air oven at 105 ^{0}C for three hours, cooled in a desiccator and the final weight was measured.

Determination of ash content

Ash content was determined by weighing 2 g of sample into a crucible and incinerating at 500 ^{0}C for three hours using a muffle furnace.

The light grey ash obtained was cooled, and the final weight of the ash content was taken.

Determination of fat content

Fat content determination was done using the Tecator Soxtec system HT 1043 extraction unit. Fat extraction was done using petroleum ether.

After extraction, the solvent was allowed to evaporate, and the fat was dried in an oven at 105 ^{0}C for one hour, cooled in a desiccator, and weighed to determine the fat content.

Determination of fiber content

To determine the fiber content. The sample was gently boiled in 200 mL of 1.25 % sulfuric acid solution for 30 minutes, the solution was filtered, washed with boiling water until the filtrate was acid free. The residue was transferred back to the beaker and gently boiled in 200 mL of 1.25 % sodium hydroxide solution, followed by filtration and washing until the filtrate was alkali-free, with a final wash using 15 ml of 95 % ethanol.

The residue was then dried in an oven at 105 ^{0}C for 2 hours, cooled in a desiccator, and weighed.

Determination of protein content

The protein content was determined by micro-Kjeldahl using the Tecator Digestion System and Kjeltec Auto 1030 Analyzer.

Kjeldahl digesting system was used to digest the sample after which it was diluted with distilled water and NaOH was added to make the solution strongly alkaline.

The released ammonia was distilled into a receiving flask containing boric acid, the distillate was titrated against standardized hydrochloric acid (0.1 M). The nitrogen content was multiplied by a conversion factor (6.25) to get protein content.

Determination of carbohydrate content

The percentage carbohydrate content of the sample was determined by adding the percentage values of ash, fat, protein, and moisture content together (% Fat + % Ash + % Protein + % Moisture) and subtracted from 100.

Statistical analysis

Values were represented as mean ± standard deviation. Mean ± standard deviation and Spearman correlation of fungi concentration against nutrient composition were determined using SPSS software. The statistical level of significance was fixed at $p < 0.05$.

RESULTS

Colony forming unit per gram of rice, maize and sorghum

The result of the proximate analysis and the colony-forming units (CFU) of fungi in rice, maize, and sorghum are presented in Tables 1, 2 & 3.

Maize had the highest fungi load, the mean value for maize was 4.0×10^5 CFU/g, and the CFU count in some maize samples was as high as 1.2×10^6 CFU/g. In contrast, rice had the lowest fungi load, with a mean of 2.7×10^4 CFU/g, with CFU count in a sample as low as 6.7×10^1 CFU/g.

The mean value of fungi load in sorghum was 3.7×10^4 CFU/g, with a range of $1.3 \times 10^4 – 1.3 \times 10^5$ CFU/g for the lowest and highest values respectively.

Effect of fungi on nutrient composition of rice, maize, and sorghum

The nutrient analysis revealed correlations between contamination and nutrient content. There was a strong positive correlation for moisture in rice (0.752), maize (0.806), sorghum (0.550) and a strong negative correlation for carbohydrates in rice (-0.638), maize (-0.860), while sorghum exhibited a relatively weak negative correlation (-0.462).

This data strongly indicates potential loss of nutrients to the fungi present.

DISCUSSION

The findings of this study highlight the significant impact of fungal contamination on the nutrient composition of rice, maize, and sorghum. The higher CFU of fungi in maize compared to rice and sorghum aligns with the work of Garba *et al.* (2017), and Onyedum *et al.* (2020), this can be attributed to its higher susceptibility to fungal contamination (Mahmoud *et al.*, 2013).

The positive correlation between moisture content and fungal presence across all three grains suggests that higher moisture levels in grains may create a favorable environment for fungal growth, which accelerates nutrient degradation. This aligns with previous research that links fungal proliferation in cereals to moisture-rich environments (Nishimwe *et al.,* 2020). The observed negative correlation between fungal contamination and carbohydrate content, particularly in maize (-0.860) and rice (-0.638), indicates that fungi consume available carbohydrates, potentially impacting the energy value of the grains for human consumption. Interestingly, while protein content showed minimal negative correlation with fungal contamination in rice, it displayed a slight positive correlation in sorghum, suggesting variability in how fungi interact with nutrient types depending on the grain. These findings underscore the need for effective grain storage solutions that limit moisture accumulation and inhibit fungal growth, as well as the potential for further investigation into grain-specific fungal resistance mechanisms.

CONCLUSION

In conclusion, this study demonstrates that fungal contamination significantly affects the nutrient composition of rice, maize, and sorghum, with notable implications for food quality and safety. The positive correlation between moisture levels and fungal growth highlights the importance of proper grain drying and storage conditions to inhibit fungal proliferation. Additionally, the depletion of carbohydrates and varying effects on protein content across the different grains emphasize the nutrient losses and potential health risks associated with consuming contaminated cereals. These findings underscore the urgent need for improved post-harvest management strategies, particularly in regions with humid climates where fungal contamination is prevalent. Future research focusing on fungal-resistant grain varieties and advanced storage solutions could further enhance food security and nutritional quality in staple cereal crops.

Table 1: Nutrient Composition and Colony Forming Units (CFU/g) of Fungi in Rice

AEZ	Crop	Location	Moisture	Ash	Fat	Fiber	Protein	Carbohydrate	Mean Fungi load. (CFU/g)
MA	RM	T1	7.16 ± 1.18	2.44 ± 0.25	0.95 ± 0.02	1.78 ± 0.19	5.37 ± 0.94	73.7 ± 0.74	3.8×10^4
MA	RS	T1	7.51 ± 1.56	2.96 ± 0.52	1.1 ± 0.16	2.00 ± 0.39	5.57 ± 0.86	75.21 ± 2.11	1.2×10^5
SGS	RM	B1	16.47 ± 2.02	9.73 ± 0.93	2.47 ± 0.11	1.16 ± 0.02	2.26 ± 0.05	63.83 ± 0.79	5.4×10^3
SGS	RS	B1	13.04 ± 1.09	9.17 ± 0.06	2.5 ± 0.10	1.19 ± 0.03	2.30 ± 0.01	61.17 ± 1.03	1.4×10^3
SHS	RM	R1	8.83 ± 0.29	3.88 ± 0.09	6.33 ± 1.89	5.83 ± 0.77	10.36 ± 1.75	63.72 ± 2.75	6.7×10^1
SHS	RS	R1	13.17 ± 1.04	2.56 ± 0.36	7.67 ± 1.76	4.86 ± 0.17	11.08 ± 1.19	59.74 ± 4.67	1.2×10^3

AEZ: Agroecological zone, MA: Mid-Altitude, SGS: Southern Guinea Savannah, SHS: Sahel Savannah, RM: Rice from Market, RS: Rice from Store

Table 2: Nutrient Composition and Colony forming units (CFU/g) of Fungi in Maize

AEZ	Crop	Location	Moisture	Ash	Fat	Fiber	Protein	Carbohydrate	Mean Fungi load. (CFU/g)
NGS	MF	B2	12.54 ± 0.47	3.10 ± 0.10	2.24 ± 0.21	3.27 ± 0.02	10.32 ± 0.02	67.37 ± 0.04	3.2×10^5
NGS	MM	B2	11.07 ± 0.06	2.92 ± 0.07	2.61 ± 0.01	3.40 ± 0.10	10.22 ± 0.02	69.28 ± 0.03	2.8×10^5
NGS	MS	B2	12.48 ± 0.02	2.97 ± 0.12	2.22 ± 0.03	3.26 ± 0.02	9.87 ± 0.01	68.45 ± 0.01	7.6×10^5
MA	MM	T1	6.15 ± 0.49	2.45 ± 0.31	1.98 ± 0.11	1.24 ± 0.24	8.10 ± 2.40	75.01 ± 5.38	1.2×10^6
MA	MS	T1	6.51 ± 0.86	2.97 ± 0.16	2.14 ± 0.19	1.46 ± 0.44	8.30 ± 2.52	76.52 ± 4.8	1.1×10^5
RF	MM	A1	10.12 ± 0.44	1.25 ± 0.05	2.33 ± 0.05	2.48 ± 0.02	7.05 ± 0.45	76.81 ± 0.71	3.7×10^4
RF	MS	A1	10.12 ± 0.26	1.26 ± 0.14	2.18 ± 0.02	2.48 ± 0.17	7.27 ± 0.49	76.68 ± 0.71	5.7×10^4

AEZ: Agroecological zone, Northern Guinea Savannah, SGS: MA: Mid Altitude, RF: Rain Forest, MM: Maize from Market, MS: Maize from Store, MF: Maize from Farm

Table 3: Nutrient Composition and Colony forming units (CFU/g) of Fungi in Sorghum

AEZ	Crop	Location	Moisture	Ash	Fat	Fiber	Protein	Carbohydrate	Mean Fungi load. (CFU/g)
MA	SM	T1	6.26 ± 0.23	1.87 ± 0.16	0.82 ± 0.13	1.74 ± 0.24	6.94 ± 0.78	75.91 ± 6.35	2.0×10^4
MA	SS	T1	6.61 ± 0.61	2.39 ± 0.25	0.98 ± 0.22	1.96 ± 0.44	7.13 ± 0.88	77.42 ± 5.76	1.7×10^4
SS	SF	A1	18.26 ± 0.31	2.07 ± 0.21	2.43 ± 0.21	3.36 ± 0.11	9.21 ± 0.22	63.98 ± 0.48	1.8×10^4
SS	SM	A1	15.53 ± 0.25	2.87 ± 0.23	2.63 ± 0.13	3.58 ± 0.10	10.43 ± 0.21	64.00 ± 0.25	1.3×10^5
DS	SF	G1	12.54 ± 0.47	3.10 ± 0.10	2.24 ± 0.21	3.27 ± 0.02	10.32 ± 0.02	67.37 ± 0.04	1.3×10^4
DS	SM	G1	11.07 ± 0.06	2.92 ± 0.07	2.61 ± 0.01	3.40 ± 0.10	10.22 ± 0.02	69.28 ± 0.03	3.2×10^4
DS	SS	G1	12.48 ± 0.02	2.97 ± 0.12	2.22 ± 0.03	3.26 ± 0.02	9.87 ± 0.01	68.45 ± 0.01	3.2×10^4
SGS	SM	M2	8.00 ± 0.00	3.30 ± 0.00	2.43 ± 0.02	3.63 ± 0.03	10.81 ± 0.02	71.25 ± 0.01	3.5×10^4
SGS	SS	M2	10.4 ± 0.10	3.17 ± 0.06	2.52 ± 0.02	3.68 ± 0.01	10.41 ± 0.02	69.21 ± 0.03	4.1×10^4

AEZ: Agroecological zone, MA: mid-altitude, SGS: Southern Guinea Savannah, DS: Derived Savannah, SM: Sorghum from Market, SS: Sorghum from Store, SF: Sorghum from Farm

Table 4: Correlation of Fungi (CFU/g) against Nutrient Composition of Rice, Maize and Sorghum

	Moisture	Ash	Fat	Fiber	Protein	Carbohydrate
Rice	0.752	-0.415	-0.584	-0.301	-0.136	-0.638
Maize	0.806	0.354	-0.488	-0.272	0.220	-0.860
Sorghum	0.550	0.258	0.413	0.390	0.384	-0.462

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OCCURRENCE OF AFLATOXIN IN THREE SELECTED SMOKED- DRIED FISH SPECIES AND ITS RISKS ON HUMAN HEALTH FROM NIGER SOUTH, NIGER STATE, NIGERIA

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ABSTRACT

Food contamination is a critical public health concern at the global level. Aflatoxins are mycotoxins produced by strains of *Aspergillus flavus* and *Aspergillus parasiticus,* from fungi as their toxic secondary metabolites. Smoke-drying of fish is a popular traditional method and smoked-dried fish is mostly consumed in Nigeria. This study aimed to evaluate the occurrence of total aflatoxins in three selected smoked- dried fish species (*Clarias gariepinus*, *Prototems annectens* and *Oreochromis niloticus*) and the extent of the threat it might pose on human health. The study was carried out from three (3) riverine communities in Niger State, comprising of katcha, Muregi and Nupeko. A total of 18 samples comprising of *Clarias gariepinus*, *Prototems annectens* and *Oreochromis niloticus* intended for human consumption were analyzed based on seasons. The lowest aflatoxin concentration value obtained is (0.103 µg/kg) of *Clarias gariepinus* from Muregi and *Clarias gariepinus* from Nupeko have the highest aflatoxin value (3.59 µg/kg). The investigation was carried out using the enzyme-linked immunosorbent assay (ELISA) Kit. All the three (3) sampling stations show the presence of aflatoxin in all the fish samples from both seasons except the samples from katcha in dry season, which showed (0µg/kg). None of the value of Aflatoxin concentration from the fish samples exceeded the maximum level permitted (4*µ*g·kg−1) by European Union, (20*µ*g·kg−1) by Joint FAO/WHO Expert Committee on food additives (JECFA) and (15µg/kg) by United State and Food and Drug Administration. The risk calculated showed that the estimated daily intake (EDI) values of Aflatoxin was found higher in children (0.175 *µ*g/kg/day, 0.196 *µ*g/kg/day and 0,413 *µ*g/kg/day) in Niger South (katcha, Muregi and Nupeko) followed by adolescent (0.098 *µ*g/kg/day, 0.110 *µ*g/kg/day and 0.232 *µ*g/kg/day) and adult (0.075 *µ*g/kg/day, 0.084 *µ*g/kg/day and 0.177 *µ*g/kg/day), the margin of exposure (MOE) values was lower in children (114.3, 102.0 and 48.4), followed by adolescent (204.1, 181.8 and 86.2) and adult (266.7. 238.1 and 113.0). All Moe values were < 10,000, which indicates possible health risk to consumers. The lower the value to 10,000, the higher the margin of exposure, the result showed children are more at risk compare to adult. According to the findings, the higher the degree of dryness in Smoked-dried fish the lower the chances of Aflatoxin contamination. Therefore, there is a need for the fishermen and the marketers to adopt a better method of preservation and storage and it will be necessary to estimate the assessment risk on infants, to understand the extent of aflatoxin contamination.

Keywords: Aflatoxin, Smoked-dried fish, *Clarias gariepinus*, *Prototems annectens*, *Oreochromis niloticus, Aspergillus.*

INTRODUCTION

Food safety and security remain a major concern in the sub-saharan Africa (Bankole *et al.*, 2006). Dry fish is the second most commonly consumed fish form in most Nigerian households and a preferred cheap method of preservation (Onyeneke *et al.,* 2020). Smoked fish products are the commonest form of fish product for consumption. Out of the total of 194,000 metric tons of dry fish produced in Nigeria, about 61% of it are smoked. One of the greatest problems affecting the fishing industry all over the world is fish spoilage, in high ambient temperature of the tropics, fresh fish have the tendency to spoil within 12 to 20 hours (Clucas and Ward, 1996). Ayeloja, *et al.,* (2018) opined that the major source of contaminants especially aflatoxins was as a result of poor handling, packaging and storage of fish and meat alike. Traditional methods are quite famous for fish processing, preservation, and storage in Nigeria, some of the most notable traditional methods used in fish processing and preservation include salting, smoke-drying and sun-drying. However, smoke-drying is the most popular method, and therefore a large volume of the fish is consumed in smoke-dried form. In rural areas where there are no refrigeration facilities, the smoke-dried fish happens to be the best option as the source of protein. The preference for the smoke-drying method lies in the fact of the availability and at low-cost of firewood. Reports have shown that mycotoxins can also occur in sun-dried or smoke-dried fish products, which are typically found in tropical and subtropical regions where high temperatures and humidity considerably influence fungal growth and toxin formation (Christane, *et al.,* 2020). Aflatoxins are mycotoxins produced by strains of *Aspergillus flavus* and *A. parasiticus*. Rao *et al.,* (2021) highlighted that they are comparatively the most known mycotoxins owing to their

ubiquitous nature. There are different types of aflatoxins, in which AFB1 and AFB2 is the most potent, among them, to both humans and animals (Agriopoulou *et al.,* 2020). Due to these predicaments posed by mycotoxins (aflatoxins), limits are usually set by countries/regions; e.g. European Union (4µg/kg), Food and Drug Administration of United State (15µg/kg) and Joint FAO/WHO Expert Committee on Food Additives (JECFA) (20µg/kg) to regulate the permissible levels to safeguard consumers from cancer attributable to Aflatoxin and some other negative health effects.

MATERIALS AND METHODS

Study Area

The study was carried out in three (3) riverine communities in Niger state comprising of Muregi in Mokwa Local Government areas (LGA), Nupeko in Lavun Local Government area and Katcha Local Government area in North Central, Nigeria. Niger State has a tropical climate consisting of two seasons, i.e., rainy season (April to September) and dry season (October to March) with annual rainfall varying from 1100 to 1600 mm (Niger State Bureau of Statistics, NSBS, 2012). The people in these communities are mostly artisanal fishermen and rice farmers, The sampling location for Muregi in Mokwa LGA is located approximately on the latitude of $8^{0}45'15"$ N and longitude $5^{0}49'58"$ E, the sampling location for Nupeko under Lavun LGA is located on the latitude $8^{0}50'41"$ N and longitude $5^{0}24'27"$ E and that of Katcha LGA is on latitude $8^{0}45'$ 38" N and Longitude $6^{0}18'$ 45" E.

Fig. 1 Map of the three (3) sample areas

Collection of fish samples

The three species of smoked dried fish (*Oreochromis niloticus, Clarias gariepinus,* and *Prototems annectens)* were sampled randomly and purchased at each market. The fishes were identified using fish chart guide and by asking fish mongers for the local name. The fish samples were collected twice for a period of eight months from (May 2023 to December 2023). The first set of the smoked-dried fish samples were collected during mid raining season (July) and the second set were collected during early dry season (December).

Bio-Shield Total 5 ELISA kit test Method

It is a quantitative test based on ELISA, it allows the user to obtain exact concentrations of Aflatoxin in part per billion (ppb) or micro-gram per kilogram (µg/kg). The wells of the microtiter strips are coated with total aflatoxin specific antibodies. Toxins are extracted from a ground sample with 70% methanol. Aflatoxin standards or samples and Aflatoxin-HRP conjugate (detection solution) are added into the coated wells. Aflatoxin-HRP conjugate binds to the binding sites of coated antibodies that are not already occupied by Aflatoxin of standard or samples. Any unbound Aflatoxin-HRP conjugate of detection solution is removed in a washing step. A chromogen substrate is added to the wells resulting in the progressive development of a blue colored complex with the detection antibody. The color development is then stopped by the addition of acid, turning the resultant final product yellow. The measurement is made photometrically at 450nm and the intensity of the produced colored complex is indirectly proportional to the concentration of total Aflatoxin present in the samples and standards (Eva Engvall and Peter Perlman, 1971).

Risk Assessment of exposure to Aflatoxin via consumption of Smoked-dried fish (Nile Tilapia, *Clarias gariepinus* and Africa Lungfish)

Estimated Daily Intake (Exposure)

The daily estimated intake (EDI) of Aflatoxin for adults, Adolescent and children were determined using the formula below and expressed in µg/kg of body weight/day (dos Santos *et al.,* 2013, EFSA, (2020).

EDI = (Daily Intake (food) x mean level of total aflatoxin)/ average body weight ……. (1)
Daily intake of smoked dried fish in Nigeria according to Assogba *et al.* (2019) is approximately 18.2g/day (6.643kg/yr). The different age categories used in this study were done as follows; children – 26 (24-28) kg (WHO, 2006, Biritwum *et al.,* 2005), adolescents – 46.25 (38.5-54) kg (Afrifa–Anane *et al.,* 2015), Adults – 60.7kg (Walpole *et al.,* 2012).

Margin of Exposure of Aflatoxin
MOE calculate the risk by the ratio of carcinogenic dose to population intake. In this study, MOE was calculated by dividing benchmark dose limit (BMDL) of total aflatoxin by toxin exposure (Adetunji *et al.,*2018) as expressed in equation below;

MOE = BMDL/Toxin Exposure (EDI) ……..…… (2).

Estimated liver cancer risk due to consumption of Smoked-dried fish
The cancer risk can be calculated using the below formula (Kortei *et al.,* 2021);

Cancer Risk = Exposure (EDI) x Average potency ……....…… (3).

According to the FAO/WHO, the population risk for primary liver cancer can be estimated with the assumption of 25% carriers of hepatitis B in developed countries (Duarte *et al.,* 2013). The potencies of hepatitis B virus (HBV) infection and HBV non-infection values are 0.3 and 0.01 respectively, estimated from animal and epidemiological studies (Duarte *et al.,* 2013). Hence, the potency of liver cancer in Nigeria population can be estimated using the following equation;

Average potency $= (0.01 \times 75\%) + (0.30 \times 25\%) = 0.0825$

Questionnaire
A total of one hundred and fifty (150) well-structured validated questionnaires of 15 items each were prepared and administered in an interview form and answered by fifty (50) fish processors each from the three communities comprising of male and female adults. An assistant field officer from the communities with at least O'Level qualification and also understood the local language helped in administering and collection of answered questionnaires.

Data Analysis
Data were analyzed using SPSS (IMB, version 29.0) packages. The mean (estimates) and standard errors of the aflatoxin levels in the various smoked-dried fish species were calculated. Significance of difference in aflatoxin levels between various fish species, the sampling area, weight, length, seasons etc. was examined using one-way variance of analysis (ANOVA). For each aflatoxin, ($p< 0.05$) mean levels between various fish species, weight, and seasons were investigated using Pearson Chi square Tests. Deterministic risk assessment models were used; dietary exposure (Estimated Dietary Intake), MOE values, Average potency and cancer risk.

RESULTS
Table 1: Concentration of Aflatoxin in smoked dried fish for both seasons.

Sample name	Sample Conc. (µg/Kg)	
	Rainy season	Dry season
KCat	0.273	0
KLung	0.295	0
KTilap	0.158	0
MCat	0.141	0.103
MLung	0.379	0.224
MTilap	0.484	0.417
NCat	0.368	3.59
NLung	0.451	0.25
NTilap	0.545	0.385

Keys: K – Katcha, M – Muregi, N – Nupeko, Cat – *Clarias gariepinus,* Lung – Lungfish, Tilap – Tilapia.

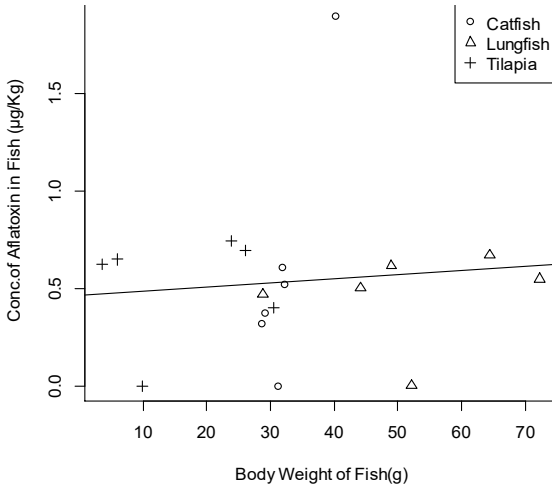

Figure 2: The relationship between aflatoxin and body weight of fishes

Table 2: Socio-demographic characteristics of the 150 surveyed consumers in the study area

	Gender		Age					Education			
Respondent	Male	Female	25-29	30-34	35-39	40-45	45 >	Primary	Secondary	Tertiary	None
(%)	48.34	51.66	4.64	35.10	27.15	17.22	15.89	50.99	45.70	1.32	1.99

Table 3: Risk Assessment for Total Aflatoxin via Consumption of Smoked-dried fish.

LGAs	Age category	Avg. Body Wgt. (kg)	EDI (µg/kg/day)	MOE	Population risk for Liver Cancer (Cases/100,000person/yr)
Katcha (0.25µg/kg)	Children (36months-10yrs)	26 (Benford *et al.* 2010)	0.175	114.3	0.014
	Adolescents (11-17yrs)	46.25 (Tongo *et al.* 2018)	0.098	204.1	8.1×10^{-3}
	Adults	60.7 (Tongo *et al.* 2018)	0.075	266.7	6.2×10^{-3}
Muregi (0.28µg/kg)	Children (36mths-10yrs)	26	0.196	102.0	0.016
	Adolescents (11-17yrs)	46.25	0.110	181.8	9.1×10^{-3}
	Adults	60.7	0.084	238.1	6.9×10^{-3}
Nupeko (0.59µg/kg)	Children (36months-10yrs)	26	0.413	48.4	0.034
	Adolescents (11-17yrs)	46.25	0.232	86.2	1.91×10^{-2}
	Adults	60.7	0.177	113.0	1.46×10^{-2}
Average					**0.014**

Margin of Exposure-MOE. Estimated Daily Intake-EDI. average potency of aflatoxin= 0.0825 Average Body weights were obtained from the different ranges referenced by the authors. 1µg = 1000ng.

DISCUSSION

The three (3) fish samples (*Oreochromis niloticus*, *Clarias gariepinus*, and *Prototems annectens*) tested, shows the presence of Aflatoxin within the acceptable range. The number of Smoked-dried fish samples contaminated with Aflatoxin is presented in Tables 1. The level of occurrence of the Aflatoxin ranged between 0 and 3.59µg/kg. The *Clarias gariepinus* from Muregi showed the lowest Aflatoxin concentration (0.103µg/kg) in dry season, *Clarias gariepinus* from Nupeko had

the highest aflatoxin concentration (3.59μg/kg) in dry season. All fish samples from rainy season shows the presence of aflatoxin except three (3) species show zero (0) aflatoxin concentration in dry season. The mean recorded 0.25, 0.28, and 0.59 μg/kg for Katcha, Muregi, and Nupeko region, respectively, and showed statistical differences ($P < 0.05$) (fig 1), when the Relationship between Aflatoxin concentration in fish samples from three regions was evaluated. Furthermore, when the effect of season on the concentration of aflatoxin was evaluated, the linear model results revealed no significant difference in aflatoxin ($P > 0.05$) between the wet and dry season and as such no seasonal effect. Despite the non-significant relationship, the Aflatoxin concentration was slightly higher in the wet season than dry season. The concentration of aflatoxin in catfish increased with an increase in body weight, while in contrary, the concentration of aflatoxin in Tilapia decreased with an increased body weight. However, the response of Lungfish to this relationship was apt, in that heavy bodied Lungfish had a decreased level of aflatoxin (Figure 2).

Occurrence of Aflatoxin

Smoked-dried fish is consumed by most Nigerians across certain age categories; children, adolescents and Adults (Bellio *et al.,* 2016). The results obtained from this study show a considerable presence of aflatoxins in Smoked-dried fish sold in Katcha, Nupeko, and Muregi markets of Niger state, Nigeria due to presence of moisture i.e the degree of dryness in the fishes were poor. Although, the concentration levels are below the permissible limit but continued exposure can have a severe effect on human health. Singh and Nsokolo, (2020), reported similar results of Aflatoxin concentration ranging from 1.3ppb – 3.84pbb when working on the prevalence of Aflatoxin on smoked-dried and fresh fish in Zambia. Akinyemi *et al.* (2011) reported much lower aflatoxin concentrations from 0.030 ppb to 1.150 ppb from smoked-dried Fish sold in Abeokuta, Ogun State, South-West Nigeria. The present study had lower aflatoxin contamination compared with previous studies on dried fish and smoked fish from Zambia and Nigeria (Kachapulula *et al.,* 2018). The high aflatoxin contamination of *Clarias gariepinus* observed in the present study may be attributed to these practices, especially poor storage and improper drying. Another reason for possible continuous high contamination observed in the rainy season values might be because of the presence of moisture (humidity) in air, which increases the chances of fungal growth and mycotoxin production. Often, retailers display the smoked-dried fish samples in trays or table openly, and this favors fungal attack and production of toxins (Eyo, 1992. Bukola *et al.,* 2008). The results obtained from the questionnaire showed that females 155 (51.7%) were more involved in smoked-drying of fish than the males with 145 (48.3%). The age of the fish processors determined their acceptability and willingness, Age range of 30 – 34years and 35- 39years had the maximum while age range of 25 – 29years and 45years>had the minimum involvement. The educational level of the fish processors has affected the improvement of traditional method to the new techniques of processing fish, fish processors with primary education and secondary education has the maximum participation with tertiary education level been the lowest. This present observation on the sex, age and educational level participation in fish processing is in accordance with report of Davies α Davies, (2009).

Risk Assessment

The estimated mean Aflatoxin 0.413 μg·kg−1·BW·day−1 (Table 4) exposure obtained from the present study was much higher than those reported in Ghana and Nigeria, with the mean dietary intake of aflatoxin in rice and peanuts (0.013 and 0.17) ng·kg−1·BW·day−1 respectively (Oyedele *et al.,* 2017, Korley Kortei *et al.,* 2019). Similarly, the estimated daily intake of aflatoxins through the consumption of smoked dried fish from this study was higher than that of Orony *et al.,* (2015).

Several studies previously used the margin of exposure (MoE) approach for risk description of genotoxic and carcinogenic mycotoxins like AFs. We adopted the same approach in this study. It is reported that an MoE value of ≥10000 should be considered as "safe," while an MoE value ≤ 10000 could cause a potential risk to public health, and the lower the value, the higher the risk (Benford *et al.,* 2010, Heshmati *et al.,* 2017, Cartus α Schrenk, 2017). As shown in Table 4.7, the MoE values obtained from this study is <10000, indicating a potential risk to the consumers of Smoked-dried fish. Previous studies reported that MoE values for the babies and toddlers were the lowest, which is not different from the present study. This indicates that children might have the highest risk of being exposed to Aflatoxin (Wang *et al.,* 2018, Zhang *et al.,*2020).

In Nigeria, cases of liver cancer have been increasing with an age standardized incidence rates (ASR) of 7.7 and 2.1 (male and female) per 100,000 population (Kew, 2013). Therefore, it is estimated from our results that consuming aflatoxin-contaminated Smoked-dried fish could be responsible for 0.18% and 0.67% representing male and female respectively, of all cancer cases of the three communities in Niger South of Niger state. This is minimal since it's not up

to 1% but it should not be ignored due to bioaccumulation or since there is a greater contribution that may take place for the population consuming a daily amount of Smoked-dried fish above the national average.

CONCLUSION AND RECOMMENDATION

Although, the presence of Aflatoxin concentrations in Smoked-dried fish was below the limits acceptable by regulatory bodies in the three different regions in Southern Niger but continuous intake or Bioaccumulation of aflatoxins contaminated food may constitute a health hazard. However, it is evident from the results of these study that smoked-dried fish could also be problematic routes for exposure to aflatoxins, since presence of moisture, improper smoking, drying and poor storage of fishes may lead to insect infestation, fungal attack and degradation of smoked-dried fish. Considering the adverse health especially hepatocellular carcinoma (HCC) outcome of the health risk assessments in different age categories since the calculated margin of exposure (MOE) were less than 10,000. It is therefore important that adopting a better method of preservation by increasing the degree of dryness and proper storage of smoked-dried fish in lessening Aflatoxin contamination in processed fish.

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Data availability: The datasets generated during or analyzed during the current study are available from the corresponding author on reasonable request.

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EFFECTS OF *ADANSONIA DIGITATA* LEAF EXTRACT ON INDOMENTHACIN INDUCED ULCER IN WISTAR RATS.

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ABSTRACT
An ulcer is a painful sore that can appear anywhere in or on the body, from the lining in the stomach to the outer layer of the skin. Current drugs used in the treatment of ulcer are effective but have side effects on prolonged use. *Adansonia digitata* is a medicinal plant used traditionally in the treatment of ulcer related diseases. The aim of this study is to screen the effects of *Adansonia digitata* leaf extract on indomethacin induced-ulcer. The phytochemical composition, acute toxicity, total acidity, pH and volume of gastric juice, *in-vivo* anti-ulcer activity and histopathology of the stomach lining were determined using standard methods. Phytochemical screenings revealed the presence of saponins, alkaloids, tannins, phenols, steroids, and flavonoids with phenols having the highest concentration (588.49±0.55 mg/100 g) followed by tannins (87.92±0.34 mg/100 g). From the acute toxicity test, none of the doses cause any form of lethality or any form of physiological abnormalities on the rats. *Adansonia digitata* show strong anti-ulcer activity with 400mg/kg BW (170.06±2.12), Total acidity 100mg/kg BW (71.87±0.76), pH 400mg/kg BW (3.68±0.18), and gastric volume 100mg/kg BW (7.56±0.19) damage respectively; and mild gastric mucosal. The highest value of 400mg/kg BW anti-ulcer activity by *Adansonia digitata* was similar to the standard (Omeprazole). This study shows that *Adansonia digitata* has strong anti-ulcer activities which is exerted through various mechanisms and can be attributed to its bioactive components.

Keywords: anti-ulcer, *Adansonia digitata*, phytochemicals.

INTRODUCTION
An ulcer is a painful sore that can appear anywhere in or on the body, from the lining in the stomach to the outer layer of the skin. Sometimes ulcers disappear on their own. Other times they require medical treatment to prevent serious complications. An example is peptic ulcer. Peptic ulcers are acid-induced lesions found in the stomach and duodenum characterized by denuded mucosa with the defect extending into the submucosa or muscularis propria (Narayanan *et al*., 2018). They are mainly of two types acute and chronic. Certain factors also affect peptic ulcers such as *Helicobacter pylori* infection, NSAID medications, stress, alcohol, smoking, diet, genetics, and hormones (Chouhan and lal., 2023).

Medicinal plants have been playing an essential role in the development of human culture. As a source of medicine, Medicinal plants have always been at forefront in virtually all cultures of civilizations. For the large proportions of world's population medicinal plants continue to show a dominant role in the healthcare system and this is mainly true in developing countries where herbal medicine has continuous history of long use. The development and recognition of medicinal and financial aids of these plants are on rise in both industrialized and developing nations (Dar *et al*., 2017).

The Bombacaceae (Baobab family) is a small family of flowering plants which contains about 28 genera and 200 species. Plants of this family are perennial, deciduous and woody trees. They occur naturally throughout the tropical and subtropical regions of the world especially in tropical America. Many species grow to become large trees, with *Ceiba pentandra* L. Gaertn. the tallest, reaching a height of 70 m (Refaat *et al.,* 2012). *Adansonia digitata* fruit, also known as baobab, has been used traditionally throughout the world for its medicinal properties. *Adansonia digitata* has antioxidant, anti-inflammatory, analgesic, and antimicrobial activities. The health benefits of *Adansonia digitata* have been attributed to its bioactive compounds, namely phenols, flavonoids, proanthocyanins, tannins, catechins, and carotenoids. *Adansonia digitata* fruit is also an important source of vitamin C and micronutrients, including zinc, potassium, magnesium, iron, calcium, and protein, which may reduce nutritional deficiencies (Silva *et al.,* 2023).

Adansonia digitata is commonly known as baobab tree native to Africa. *It belongs to the Bombacaceae* family. The binomial name of *Adansonia digitata* was given by Linnaeus, the generic name honouring Michel Adanson who had been to Senegal in the eighteenth century to describe baobab (Rahul *et al*., 2015). It is a large, conspicuous tree, native to semiarid regions of Africa, Asia (in China and Malaysia), Australia, and the Caribbean (especially Jamaica). Locally called *Dima* (in

Tigrinya), the plant is very common in deserts and hot lowlands in Ethiopia. The tree is highly valued by rural communities of arid and hot lowlands of sub-Saharan Africa for its fruits and edible leaves (Wasihun *et al.,* 2023).

MATERIALS AND METHODS

Plant collection

Adansonia digitata leaves were acquired from the vicinity of the Nigerian railway station, Shakwata, Minna in the Bosso LGA of Niger state, Nigeria. They were air-dried at room temperature and then crushed into a powder using a mortar and pestle. The leaves were stored in a neat container with labelled information when not being used for analysis.

Experimental animals

Wistar rats, both sexes, were sourced from Murine Top Farms, Minna, Niger State. These rats were kept in a well-ventilated plastic cage and given commercial rat food and water as needed. The study was carried out at the Department of Biochemistry Animal House, Federal University of Technology, Minna, according to the recommendations provided by the Canadian Council on Animal Care. All studies were conducted in accordance with international standards for the use and care of laboratory animals.

Reagents and Chemicals

All chemicals used for this study were of analytical grade.

Extraction of plant samples

Cold maceration method of extraction was used for the extraction of the plant samples. Approximately 100 g of the plant sample was weighed into a clean 2000 mL conical flasks, 1000 mL of methanol was added and allowed to stand at room temperature for 72 hours with constant shaking using shaker. After 72 hours, the extracts was filtered into clean beaker using Whatman filter paper. The extract was air-dried to a constant weight. The dried extract was then kept in an air-tight container until for further use.

Phytochemical analysis

Qualitative and quantitative phytochemical analysis were carried out to determine the presence of phenol, flavonoid, alkaloid, saponins, Tannin content (AOAC, 2005).

In-Vivo Studies

Acute Toxicity Test

The acute toxicity of the extract was evaluated according to OECD guideline 423 using the acute toxic class technique. Three groups of three rats each were administered oral doses of the extract at 10, 100 and 1000 mg/kg in the first phase and at 1,500, 2,900 and 5,000 mg/kg in the second phase. Toxic and fatalities effects on the rat were monitored, and by using the lowest fatal dosage and the greatest non-lethal dose, the LD_{50} was calculated.

Experimental Design

Eighteen (18) rats of both sex weighing between 73-156g were grouped into six (6) groups of three (3) rats each, labelling from groups I-VI

Group I: 100mg/kg of extract was administered

Group II: 200mg/kg of extract was administered

Group III: 400mg/kg of extract was administered

Group IV: Positive control (indomethacin)

Group V: Negative control (NC)

Group VI: Omeprazole (Standard)

Indomethacin-Induced Ulcer Model

The ulcer was induced with indomethacin at a dose of 18 mg/kg to evaluate the ulcer healing effect of the plant extract which was compared with the NC (vehicle) and standard (Omeprazole 10 mg/kg) treated groups. The treatment groups received 100, 200, 400 mg/kg (once daily) for 21 days. The first dose was given 6 hours after induction of ulcer with indomethacin (18 mg/kg) (Inas *et al.,* 2011).

Determination of pH and Gastric Volume

An aliquot of gastric juice was taken and the pH of the solution was measured using a pH meter based on the method of Jalilzadeh-Amin *et al.* (2015). The volume of gastric juice of each animal was measured after centrifugation with 1000 rpm for 10 minutes and analyzed since it is one parameter for the study of the antisecretory effect of the plant extract.

Determination of Total Acidity

An aliquot of 1 ml gastric juice diluted with 9 ml of distilled water was taken and two drops of phenolphthalein indicator were added. Then, it was titrated with 0.01 N NaOH until a permanent pink color was observed. Based on the volume of 0.01 N NaOH consumed, the total acidity was expressed as mEq/l by the following formula (Jalilzadeh-Amin *et al.,* 2015):

Total Acidity = Vol. of NaOH × N × 100 mEq/l/0:1

Histology of the gastric tissue

For the histological analysis of the gastric tissues, a section of the tissue was fixed in neutral buffered formalin solution (10%) immediately after excision from the animals. The conventional paraffin embedding technique (dehydration by ascending grades of ethanol, using chloroform for clearing and embedding with paraffin wax at 60 ºC) was employed in processing of the fixed tissues. Sections of 3-4 μm thick were obtained and stained with haematoxylin and eosin from prepared paraffin blocks. Under light microscope (magnification X40), the histology observations were made.

Statistical Analysis

The mean ± SEM of three replicate analyses was used to express all values. The statistical analysis was carried out using SPSS statistical package for WINDOWS (version 23.0; SPSS Inc, Chicago). The data was analyzed by ANOVA and the significance level was set at $p<0.05$.

RESULTS

Qualitative phytochemical compositions of *Adansonia digitata*

Table 1 and 2 revealed the results of the qualitative and quantitative phytochemical analysis of *Adansonia digitata*. The plant was found to contain various secondary metabolites including phenols, flavonoids, alkaloids, tannins, and saponins.

Table 1: Qualitative phytochemical screening of *Adansonia digitata* leaf extract

Phytochemical	Bioavailability
Phenols	+
Flavonoids	+
Tannins	+
Saponins	+
Alkaloids	+

Key: (+) present

Table 2: Quantitative Phytochemical screening of *Adansonia digitata* leaf extract

Phytochemical	Concentration (mg/100 g)
Phenols	588.49 ± 0.55^{e}
Flavonoids	49.86 ± 0.25^{b}
Tannins	87.92 ± 0.34^{d}
Saponins	57.58 ± 0.37^{c}
Alkaloids	16.96 ± 0.21^{a}

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts along column are significantly different at $p < 0.05$.

Acute Toxicity Test (LD_{50}) of *Adansonia digitata* leaves

There was no lethality or behavioral change in the three groups of mice that received 10,100 and 1000mg/kg body weight of the extract. Also, higher doses of 1600, 2900 and 5000 mg/kg body weight of the extract to three other groups showed no death or behavioral change occurred within 72 hours of administration.

Table 3: Lethal dose of *Adansonia digitata* leaves extract

Phases	Dose administered (mg/kg Body Weight)	Signs of toxicity	Mortality
Phase I			
Group 1	10	Nil	0/3
Group 2	100	Nil	0/3
Group 3	1000	Nil	0/3
Phase II			
Group 1	1600	Nil	0/3
Group 2	2900	Nil	0/3
Group 3	5000	Nil	0/3

pH, Gastric Volume and Total Acidity

Table 4. displays the pH, gastric volume and total acidity value of *Adansonia digitata* leaf extract and Omeprazole (Standard). The values varied based on concentration.

Table 4: effect of *Adansonia digitata* leaf extract on pH, gastric content and total acidity of the various groups of rats.

Sample	pH	Gastric volume (mL)	Total acidity (meq/L)
Negative control	1.59 ± 0.15^{a}	8.88 ± 0.38^{d}	83.79 ± 0.93^{e}
Positive control	3.44 ± 0.18^{c}	5.65 ± 0.13^{a}	47.38 ± 1.25^{a}
Omeprazole (Standard 10mg/kg)	3.55 ± 0.13^{c}	6.90 ± 0.18^{bc}	56.37 ± 1.19^{b}
400mg/kg B.W	3.68 ± 0.18^{c}	6.24 ± 0.15^{ab}	55.24 ± 1.28^{b}
200mg/kg B.W	3.39 ± 0.15^{c}	6.34 ± 0.12^{b}	60.87 ± 0.76^{c}
100mg/kg B.W	2.54 ± 0.13^{b}	7.56 ± 0.19^{c}	71.87 ± 0.76^{d}

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts along column are significantly different at $p < 0.05$.

Anti-ulcer activity of *Adansonia digitata* leaf extract

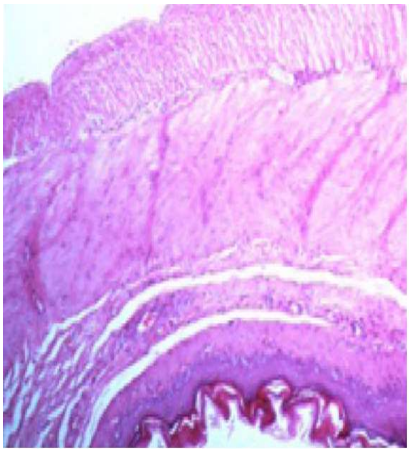
Table 5 presents the anti-ulcer activity for both the methanol extract of *Adansonia digitata* and Omeprazole (Standard) at different concentrations. *Adansonia digitata* extract caused a concentration dependent increase in anti-ulcer activity, increasing the anti-ulcer activity from 132.06 ± 4.22^{a}, 135.37 ± 3.94^{a}, and 128.20 ± 4.93^{a} at the concentration of 100mg/kg B.W, 200mg/kg B.W and 400mg/kg B.W to 148.86 ± 5.30^{b}, 154.76 ± 6.29^{b}, 170.06 ± 2.12^{c}, respectively while Omeprazole (Standard) had 193.81 ± 3.29^{d} at the concentration of 10mg/kg. The anti-ulcer activity increases with increasing concentration.

Table 5: The anti-ulcer effect of *Adansonia digitata* leaf extract on indomethacin-induced gastric ulcers in Wistar rats.

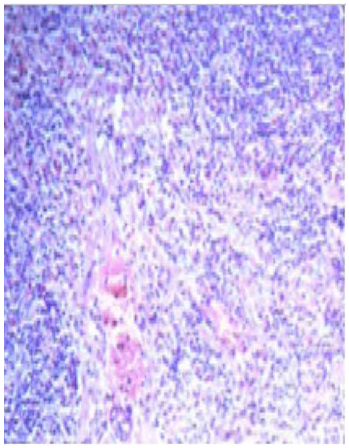
Sample	Week 0	Week 1	Week 2	Week 3
Negative control	140.42 ± 2.28^{a}	135.75 ± 1.65^{a}	130.81 ± 1.26^{a}	128.42 ± 0.86^{a}
Positive Control	137.14 ± 5.17^{a}	159.17 ± 2.96^{b}	175.71 ± 3.03^{d}	193.81 ± 3.29^{d}
Omeprazole (Standard 10mg/kg)	129.46 ± 4.10^{a}	140.41 ± 4.55^{a}	158.62 ± 3.91^{c}	178.89 ± 3.51^{c}
400mg/kg B.W	128.20 ± 4.93^{a}	140.17 ± 2.14^{a}	155.36 ± 1.52^{c}	170.06 ± 2.12^{c}
200mg/kg B.W	135.37 ± 3.94^{a}	142.27 ± 3.85^{a}	150.31 ± 2.79^{bc}	154.76 ± 6.29^{b}
100mg/kg B.W	132.06 ± 4.22^{a}	136.16 ± 4.57^{a}	142.29 ± 5.53^{b}	148.86 ± 5.30^{b}

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts along column are significantly different at $p < 0.05$.

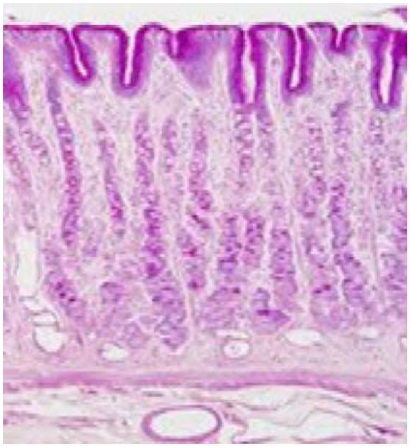
Histopathology of the stomach lining



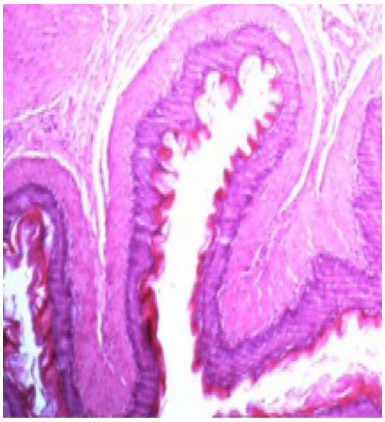
Normal control



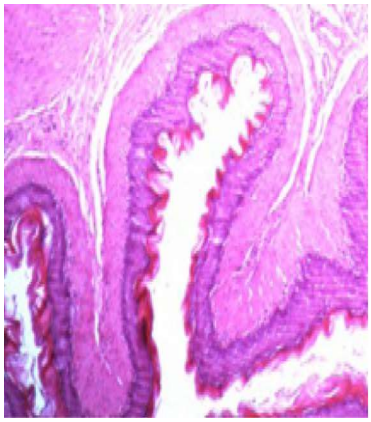
Positive control



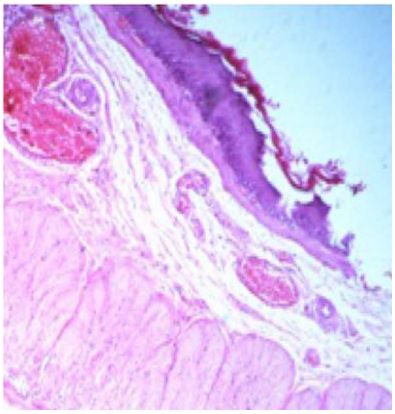
Standard control



100 mg/kg BW *A. digitata*



200 mg/kg BW *A. digitata*



400 mg/kg BW *A. digitata*

DISCUSSION

The phytochemical analysis of *Adansonia digitata* extract revealed the presence of various secondary metabolites, including saponins, flavonoids, tannins, and alkaloids. These metabolites are known to possess various bioactive properties, such as anti-inflammatory, antioxidant, antimicrobial, anticancer and antiulcer activities. Quantitative analysis of the phytochemicals showed that the extract was particularly rich in phenols, and tannins, with levels of 588.49±0.55 and 87.92±0.34 respectively. Phenols have diverse biological activities such as anti-inflammatory, anticancer, anti-aging, antibacterial, and antiviral activities. Dietary polyphenols have been used to prevent and treat allergy-related diseases. The chemical and biological contributions of phenolic compounds to ulcer have also been described (Rahman *et al.,* 2022). Tannins have been used throughout history for their pharmacological properties as part of plants and herbs in traditional medicine. They also play vital role in protecting plants against fungi and insects (Szczurek, 2021). The extract also contains relatively high levels of saponins (57.58±0.37) which are known for their biological activities such as: anticancer, antimicrobial, antiprotozoal, delivery adjuvant, anti-cholesterol, and anti-inflammatory properties (Kaur *et al.,* 2024). Flavonoids were also present in significant amounts (49.86±0.25), and these compounds have anti-oxidative, anti-inflammatory, anti-mutagenic, antiviral, antiulcer, antimicrobial, anticancer, cardioprotective, neuroprotective effects and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function (Panche *et al.,* 2016). Alkaloids, another class of secondary metabolites, were also present in the extract (16.96±0.21), they play an essential role in both human medicine and in an organism's natural defense (Heinrich *et al.,* 2021). The presence of these secondary metabolites in *Adansonia digitata* extract suggests that the plant may have potential for the development of natural remedies and pharmaceuticals.

Determination of LD_{50} is usually the first step in the toxicological evaluation of medicinal plants. The absence of mortality at the dose of 5000mg/kg bodyweight of aqueous seeds extract of *Adansonia digitata* in rats showed that the

extract is non-toxic acutely. The zero-mortality rate suggests that the administered dose (5000mg/kg bodyweight) is below the lethal dose (LD50) of *Adansonia digitata* leaf extract in Wistar rats. This finding aligns with the Organization for Economic Cooperation and Development (OECD) guidelines, categorizing substances with LD50 > 5000mg/kg bodyweight as Class 5, the lowest toxicity class (OECD, 2017).

Gastric juice parameters (pH, gastric volume and, total acidity) collectively provide valuable insights into the physiological and pathological processes occurring within the stomach. They are valuable indicators of gastric function and health. A normal gastric pH ranges from 1.5 to 3.5, with values exceeding 4 indicating hypochlorhydria and those below 1.5 suggesting hyperchlorhydria (Singh and Kumar 2017). In indomethacin-induced ulcers, gastric pH plays a pivotal role, as increased acidity worsens mucosal damage. The negative control group showed a significantly lower gastric pH (1.59 ± 0.15) compared to the normal control group (3.44 ± 0.18), indicating increased acidity in the stomach due to indomethacin-induced ulcer. The omeprazole treatment group showed a slightly higher gastric pH (3.55 ± 0.13) compared to the normal control group. The *Adansonia digitata* leaf extract treatment groups showed a dose-dependent increase in gastric pH, with the 400mg/kg group showing a significant increase (3.68 ± 0.18) compared to the negative control group. The 200mg/kg and 100mg/kg groups showed moderate increases in gastric pH (3.39 ± 0.15 and 2.54 ± 0.14, respectively).

Total acidity, shows the concentration of hydrogen ions in gastric juice. Normal total acidity ranges from 20 to 100 mEq/L (Adeyemi *et al*., 2022). Elevated total acidity adds to gastric ulcer development. The negative control group showed a significantly higher total acidity (83.79 ± 0.93) compared to the normal control group (47.38 ± 1.25), indicating increased acid production due to indomethacin-induced ulcer. The omeprazole treatment group showed a moderate decrease in total acidity (56.37 ± 1.19) compared to the negative control group. The *Adansonia digitata* leaf extract treatment groups showed a dose-dependent decrease in total acidity (Kumar *et al.,* 2023). The 400mg/kg group showed a significant decrease (55.24 ± 1.28), while the 200mg/kg and 100mg/kg groups showed moderate decreases (60.87 ± 0.76 and 71.87 ± 0.76, respectively). The results demonstrated the potential benefits of *Adansonia digitata* leaf extract, particularly at the 400mg/kg dose in mitigating indomethacin-induced ulcer in Wistar rats, as evidenced by improved gastric pH, reduced gastric volume, and decreased total acidity.

The stomach lining (gastric mucosa), plays an important role in protecting the stomach from the corrosive effects of gastric acid and enzymes (Kumar *et al.,* 2023). The histopathological examination of the stomach lining shows significant differences among the treatment groups. The negative control group showed severe gastric mucosal damage, ulceration, and erosion, indicative of indomethacin-induced ulcer, which align with the findings of American Gastroenterological Association (2020). The normal control group demonstrated normal gastric mucosal architecture, confirming the absence of ulcerative stress. The omeprazole group showed mild to moderate gastric mucosal damage, indicating partial protection against indomethacin-induced ulcers. The *Adansonia digitata* leaf extract treatment groups demonstrated dose-dependent protection against gastric mucosal damage. The 400mg/kg bodyweight group showed mild gastric mucosal damage, indicating significant protection. The 200mg/kg bodyweight and 100mg/kg bodyweight groups showed moderate and moderate to severe gastric mucosal damage, respectively.

The results of the anti-ulcer activity suggests that *Adansonia digitata* extract possesses significant antiulcer activity and the level of activity is concentration-dependent. The 400mg/kg B.W value in week 3 of 170.06 ± 2.12^{c} indicates that *Adansonia digitata* extract has a strong antiulcer ability. The results demonstrate the potential of *Adansonia digitata* extract as the natural source of antiulcer. Additionally, the near significant difference between the effect produced by the standard drug (Omeprazole) and that of the extract suggests that *Adansonia digitata* extract may have relevance in the management of ulcer disorders.

CONCLUSION

The methanol extract of *Adansonia digitata* showed significant anti-ulcer activity. The study shows that the anti-ulcer activities of *Adansonia digitata* is concentration-dependent and can be attributed to its bioactive components

RECOMMENDATION

It is recommended that further studies be carried out to isolate and purify the constituents involved in the anti-ulcer activities of this plant, structural elucidation of the active constituent(s), structure activity relationship with a view to modify the active agent to obtain more potent agent (by synthesis) and evaluation of the potential usefulness of this plant extract in the management of clinical conditions associated with ulcer.

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PROXIMATE, MINERAL, AND PHYTOCHEMICAL COMPOSITION OF METHANOL EXTRACT OF *URTICA DIOICA* LEAF AS A POTENTIAL MYCOTOXIN COMBATANT

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ABSTRACT
This study investigated the proximate, mineral, and phytochemical composition of *Urtica dioica* (stinging nettle) leaf extract to assess their potential as a natural combatant against mycotoxin. The analyses were carried out using the Association of Official Analytical Chemists (AOAC) standards. The phytochemical analysis of methanolic leaf extracts revealed a high concentration of total phenolics (58.3 mg/g) and flavonoids (42.5 mg/g), moderate levels of saponins (15.6 mg/g) and tannins (12.4 mg/g), alongside lower concentrations of alkaloids (6.8 mg/g), and steroids (3.2 mg/g). Proximate analysis showed a composition of 9.70% moisture, 3.33% of ash, 12.45% protein, 3.00% fibre and 65.66% carbohydrates. The mineral analysis highlighted significant levels of calcium (110.5%), magnesium (24.7%), iron (15.2%), and zinc (3.4%). The high magnesium and zinc levels suggest a capacity for immune modulation and oxidative stress reduction, enhancing resilience against AFB1 toxicity. The extract of *Urtica dioica* diverse phytochemicals, nutrient profile, and mineral richness accentuated its therapeutic potential as a functional food, which may serve as a dietary supplement with applications in reducing mycotoxin-related health risks, emphasizing their dual role in nutrition and bioactive compound provision.

Keywords: Stinging nettle, antioxidant, mitigation, physiological functions, supplement.

INTRODUCTION
The increasing prevalence of food contamination by mycotoxins presents a significant challenge to food safety, public health, and the agricultural economy. Mycotoxins are toxic secondary metabolites produced by certain fungi, which commonly contaminate staple foods, grains, nuts, and animal feeds, particularly under favourable environmental conditions such as high humidity and temperature. Notably, aflatoxins, ochratoxins, fumonisins, and zearalenone are among the most dangerous mycotoxins, linked to severe health issues, including immunosuppression, carcinogenicity, hepatotoxicity, nephrotoxicity, and teratogenicity (Sun *et al*., 2023; Pallarés *et al*., 2022). The socio-economic impact of mycotoxin contamination is particularly severe in developing countries, where food quality control measures may be less stringent and reliance on vulnerable crops is higher (Marc, 2022; Dlamini *et al*., 2022). This situation underscores the urgent need for safe, effective, and affordable mycotoxin mitigation strategies to safeguard food security and public health (Ong *et al*., 2023; Ortega-Beltran and Bandyopadhyay, 2023).

Traditional methods for controlling mycotoxin contamination, such as chemical preservatives, while effective, may pose health risks and face limited consumer acceptance due to potential adverse effects (Ong, 2023). Physical methods, including thermal processing, are often only partially effective since many mycotoxins are heat-stable (Sheng *et al*., 2022). In recent years, there has been a growing interest in natural, plant-based alternatives due to their biocompatibility, relatively low toxicity, and potential for sustainable production (Marc, 2022). One plant that has gained attention for its bioactive properties is *Urtica dioica*, commonly known as stinging nettle. This plant has been utilized in traditional medicine and as a food source, being rich in a variety of phytochemicals, minerals, and nutrients, making it a promising candidate for addressing food safety concerns, particularly as a mycotoxin antagonist (Ong *et al*., 2023).

One of the most compelling aspects of *Urtica dioica* lies in its diverse phytochemical composition, which includes polyphenols, flavonoids, alkaloids, tannins, and vitamins, all of which are associated with strong antioxidant, anti-inflammatory, and antimicrobial properties. Research indicates that *Urtica dioica* extracts exhibit significant antioxidant activity, which is crucial in mitigating the harmful effects of oxidative stress associated with mycotoxin exposure (Flórez *et al*., 2022). Given the safety profile and nutritional benefits of *Urtica dioica*, there is significant potential for incorporating this plant into agricultural practices aimed at reducing mycotoxin contamination in crops and animal feeds. For instance, nettle-based feed additives could improve animal health and reduce mycotoxin bioaccumulation in livestock products, leading to safer meat, milk, and eggs for human consumption (Albogami, 2023). Its rich composition of proteins, minerals, fibre, and phytochemicals provides not only nutritional benefits but also potential protective effects against

mycotoxins. By understanding the proximate, mineral, and phytochemical composition of *Urtica dioica*, researchers and food safety professionals can further explore its applications as a natural remedy in combating mycotoxin contamination, advancing both public health and food security. This paper aims to detail the proximate, mineral, and phytochemical composition of *Urtica dioica* leaves, highlighting its potential as a functional food and natural mycotoxin antagonist and paving the way for future research and practical applications in food and feed safety.

MATERIALS AND METHODS

Sample collection

Fresh leaves of *Urtica dioica* was collected from Jos, Plateau State. The plant was further identified and authenticated at the department of Botany, University of Nigeria, Nsukka, Enugu State, Nigeria.

Sample preparation

Preparation of methanol leaf extract

Exactly 500 g of the plant sample was thoroughly cleaned and ground into powder. The powdered sample was placed in a container covered with methanol in a ratio 1:10 (w/v) plant material to methanol (500 g in 5 liters). The mixture was allowed to soak for 48 hours at room temperature with occasional agitation. The mixture was then filtered to separate solid plant material from liquid extract. The extract was then concentrated using a rotary evaporator, ensuring the removal of methanol. The extract was then air-dried to remove any remaining solvent and moisture. It was then weighed and stored in an airtight container for further analysis.

Phytochemical analysis

The quantitative analysis of the phytochemical constituents of the extract were performed using the standard methods as described by the Association of Official Analytical Chemists (AOAC) (2015).

Determination of alkaloids

A sample of 2.5 g of dried plant material was subjected to repeated extraction using 100 ml of 80% methanol solution at room temperature. The extract was then filtered through the Whatman No. 42 filter paper (125 mm). The filtrate was transferred into a crucible, where it was evaporated to dryness on a water bath, and the residue was weighed.

Determination of phenolic content

Plant extracts were prepared by dissolving them in methanol to achieve a concentration of 500 μg/ml. In a test tube, 0.1 ml of the extract was combined with 3.9 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent, and the mixture was allowed to incubate at room temperature for 3 minutes. Following this, 2 ml of 20% sodium carbonate was added, and the solution was heated in a boiling water bath for one minute. The resulting blue colour was read at 650 nm. Each experiment was conducted in triplicate. A calibration curve was constructed, using gallic acid (100-500 μg/ml) as standard and the total phenolic content of the extract (μg/ml) expressed as gallic acid equivalents.

Determination of saponins

About 0.5 g of the extract was added into 20 ml of M hydrochloric acid and boiled for 4 hours. After cooling, it was filtered and 50 ml of petroleum ether was added to the filterate and evaporated to dryness. Five millilitres (5 ml) of ethanol were added to the residue and 0.4 ml was taken into 3 different test tube. 6 ml of ferrous sulphate reagent was added and 2 ml of concentrated H2SO4 was added after. After 10 mins, it was thoroughly mixed and the absorbance was read at 490 nm (Obadoni and Ochuko, 2002).

Determination of flavonoids

The plant extract was prepared by dissolving them in methanol to achieve a concentration of 500 μg/ml. In a 10 ml volumetric flask, 1 ml of the test sample was combined with 4 ml of distilled water and allowed to stand for five minutes. Following this, 0.3 ml of 5 % sodium nitrite and 0.3 ml of 10 % aluminum chloride hexahydrate were added. The mixture was then incubated at room temperature for six minutes. After incubation, 2 ml of 1 M NaOH was added to the reaction mixture. Immediately, the final volume was made of 10 ml of distilled water. The absorbance was measured at 510 nm. A calibration curve was established using quercetin standards ranging from 100 to 500 μg/ml, with the total flavonoid content of the extract expressed in μg/ml as quercetin equivalents.

Determination of tannin

Two (2 mL) Eppendorf tubes were filled with 100 mg of poly(vinyl polypyrrolidone) (PVPP). 500 μL of distilled water and the plant sample were added and incubated for 4 h at 4 °C. The Eppendorf tubes were centrifuged at 3000 rpm for 10 min at 4 °C following incubation. Only the nontannin phenolics are present in the supernatant.

The Folin–Ciocalteu technique, with a few adjustments, was used to calculate the tannin content. A 10 mL volumetric flask was filled with distilled water, and 0.1 mL of non-tannin phenolics extract was added. Dilution with distilled water brought the final volume to 4 mL after adding 0.5 mL of (1 N) Folin–Ciocalteu reagent and 2.5 mL of 5% sodium carbonate solution.

Standard tannic acid dilutions (2.5, 5.0, 7.5, 10.0, and 12.0 mg/mL) were made in the same way described above. After vigorous shaking, the mixture was incubated at 30 °C for 40 min.

The absorbances of reference and test solutions were measured with a blank UV–visible spectrophotometer at 725 nm. The non tannin concentration was represented as tannic acid equivalents per gram of dry extract. The blank solution was prepared in a similar way as explained above.

The sample's tannin content was then determined using the following equation:

tannins (g) = total phenolics (g) – non tannin phenolics (g)

Determination of steroids

Exactly 0.1 g of the extract was dissolved in a distilled water and 1 ml of the extract was transferred into 10 ml volumetric flask. Thereafter 2 ml of sulphuric acid (4 N) and 2 ml of 0.5 % iron (III) chloride was added, followed by 0.5 ml of 0.5 % potassium hexacyanoferrate (III) solution. The mixture was heated in a water bath maintained at 70 ± 20 ^{o}C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 750 nm against the reagent blank.

Proximate analysis

The methods described by AOAC (2020) were adopted for the determination of moisture, ash, crude lipid, crude protein, crude fibre and carbohydrate.

Determination of moisture content

A clean crucible was dried to constant weight in an air oven at 105^{o}C, cooled in a desiccator and weighed (W_1). Two grams of sample were accurately weighed into the previously labeled crucible and reweighed (W_2). The crucible was dried in oven to a constant weight (W_3). The percentage moisture content was calculated thus:

$$\% \text{ Moisture content} = \frac{Weight\ of\ water}{Weigh\ \ of\ dry\ sample} \times\ 100$$

Determination of ash content

The porcelain crucible was dried in an oven at 100 ^{o}C for 10 minutes, cooled in a desiccator and weighed (W_1). Two grams of the sample was placed into the previously weighed porcelain crucible and weighed (W_2). The sample was first ignited and transferred into a furnace, which was then set at 550 ^{o} C. The sample was left in the furnace for eight hours to ensure proper ashing. The crucible containing the ash was then removed cooled in the desiccator and weighed W_3. The percentage ash content was calculated as:

$$\% \text{ Ash content } = \frac{W3 - W1}{W2 - W1} \times\ 100$$

Determination of crude lipid content

A clean, dried 500ml round bottom flask containing a few anti-bumping granules was weighed (W_1) and 300ml of Petroleum ether (40-60 ^{o}C) for extraction was poured into the flask fitted with a soxhlet extraction unit. The extractor thimble containing twenty grams of the sample was fixed into the soxhlet extraction unit. The round bottom flask and a condenser were connected to the soxhlet extractor, and cold-water circulation was applied. The heating mantle was switched on, and the heating rate was adjusted until the solvent refluxed at a steady rate. Extraction was carried out for six hours. The solvent was recovered and the oil was dried in the oven at 70 ^{o}C for one hour. The round bottom flask containing the oil was cooled in the desiccator and then weighed W_2.

The lipid content was calculated thus;

$$\% \text{ Crude Lipid Content } = \frac{W2 - W1}{\text{Weight } of\ sample} \times\ 100$$

Determination of crude fibre content

Two grams of sample was weighed out into a round bottom flask. 100 ml of 0.25 M Sulphuric acid Solution was added and the mixture boiled under reflux for 30 min. The hot solution was quickly filtered under sunction. The insoluble

matter was washed several times with hot water until it was acid free. It was quantitatively transferred into the flask and 100ml of hot 0.31M Sodium hydroxide solution was added and the mixture boiled again under reflux for 30 minutes and quickly filtered under sunction. The insoluble residue was washed with boiling water until it was based-free. It was dried to constant weight in the oven at 100 ^{o}C, cooled in a desiccator and weighed (C_1) was then incinerated in a muffle furnace at 550 ^{o}C for 2 h, cooled in the desiccator and reweighed (C_2).

$$\text{The loss of weight on incineration} = \frac{C2 - C1}{\text{Weight } of\ original\ sample} \times 100$$

Determination of nitrogen and crude protein

Protein digestion

Exactly 1.5 g of the defatted sample in an ashless filter paper was dropped into 300 ml Kjeldahl flask. Twenty-five milliliters of H_2SO_4 and 3 g of digesting mixed catalyst (weighed separately into an ashless filter paper) were dropped into the Kjeldahl flask. The flask was then transferred to the Kjeldahl digestion apparatus. The sample was digested until a clear green colour was obtained. The digest was cooled and diluted to 100 ml with distilled water.

Distillation of the digest

About 20 ml of the diluted digest was measured into a 500 ml Kjeldahl flask containing anti-bumping chips and 40 ml of 40 % NaOH was slowly added by the side of the flask. A 250 ml conical flask containing a mixture of 50 ml of 2 % Boric acid and 4 drops of mixed indicator was used to trap the ammonia liberated. The conical flask and the Kjeldahl flask were then placed on the Kjedahl distillation apparatus, with the tubes inserted into the conical flask and the Kjedahl flask. The flask was heated to distill out NH_3 evolved. The distillate was collected into the boric acid solution. From the point when the boric acid turned green, 10 minutes were allowed for complete distillation of the ammonia present in the digest. The distillate was then titrated with 0.1 M HCl.

Calculation:

$$\%\ \text{N} = \frac{14 \times \text{M} \times \text{Vt} \times \text{Tv} \times 100}{\text{Weigh } of\ Sample\ (mg)} \times \text{Va}$$

$$\%\ \text{Crude protein} = \%\ \text{Nitrogen}\ (N_2) \times 6.25$$

Where; M = Actual molarity of acid

T_v = Titre volume of HCl used

V_t = Total volume of diluted digest

V_a = Aliquot volume distilled

Determination of carbohydrate

The total carbohydrate content was determined by difference. The sum of the percentage of moisture, ash, crude lipid, crude protein and crude fibre was subtracted from 100 (Muller and Tobin, 1980).

Calculation:

$$\%\ \text{Total Carbohydrate} = 100 - (\%\ \text{Moiture} + \%\ \text{Ash} + \%\ \text{Fat} + \%\ \text{Protein} + \%\ \text{Fibre})$$

Mineral composition

The mineral contents were analysed using S240 varian atomic absorption spectrophoto meter, equipped with nitrous oxide oxidant gas, acetylene gar, air oxidant gas and distilled water.

Sample Digestion

Exactly 2 g was weighed out of the dried sample into a digestion flask and 20 ml of the acid mixture was added (650 ml conc. HNO_3) 80 ml per choric acid, 20 ml concentrated H_2SO_4. Heated the flask until a clear digest was obtained. the digest was diluted with distilled water to the 25 ml mark. Appropriate dilutions were made for each element.

Procedure

The sample was thoroughly mixed by shaking, and 100 ml of it transferred into a glass beaker of 250 ml volume. The sample was prepared into the oxidizing air-acetylene flame. When the sample was aspirated, the sensitivity for 1 % adsorption was observed.

RESULTS

Phytochemical Constituents

Table 1 shows the quantitative results of methanolic leaf extract of *Urtica dioca,* which shows a high concentration of total phenolics (58.3 ± 2.5 mg/g) and total flavonoids (42.5 ± 1.2 mg/g). Saponins (15.6 ± 1.1 mg/g) and tannins (12.4 ± 0.8 mg/g) are present in moderate amounts. Alkaloids (6.8 ± 0.5 mg/g), terpenoids (5.1 ± 0.6 mg/g), and steroids (3.2 ± 0.3 mg/g) are present in lower quantities.

Table 1. Quantitative phytochemical composition of methanol extract of *Uritica dioca* leaf

Phytochemical	Quantity (mg/g of Extract)
Total Flavonoids	42.5 ± 1.2
Total Phenolics	58.3 ± 2.5
Saponins	15.6 ± 1.1
Tannins	12.4 ± 0.8
Alkaloids	6.80 ± 0.5
Steroids	3.20 ± 0.3

Proximate composition

The proximate analysis of *Urtica dioica* leaf extract in Table 2 showed that the sample contains 9.70 % moisture, 3.33 % ash content, a high crude protein content of 12.45 %, and crude fibre content of 3.00 %, while crude lipid content is 5.88%, carbohydrates constitute the largest portion at 65.66 %.

Table 2. Proximate composition of methanol extract of *Uritica dioca* leaf

Parameters	Percentage Values (%)
Moisture content	9.70^{b} ± 0.24
Ash content	3.33^{a} ± 0.00
Crude protein	12.45^{c}± 0.02
Crude fibre	3.00^{a} ± 0.02
Crude lipid	5.88^{b} ± 0.02
Carbohydrate	65.66^{d} ± 0.26

Values are given as mean ± SD of two runs of experiments. Values with the same superscript are not significantly different at $p \leq 0.05$

Mineral composition

The mineral composition of *Urtica dioca* (*U. dioca*) leaf extract shown in Table 3 reveals a variety of essential minerals present in significant amounts. (Table 4.3). Iron (Fe) is notably abundant at 15.2 %. zinc (Zn) is present at 3.4 %, manganese (Mn) and copper (Cu) are also present at 2.5 % and 1.2 %, respectively. Magnesium (Mg) is found at a high level of 24.7 %, while calcium (Ca) is the most abundant mineral in the extract at 110.5 %, sodium (Na) is present at 20.3 %.

Table 3. Mineral composition of methanol extract of *Urtica dioica* leaf

Minerals	Percentage Values (Dry weight Basis)
Fe	15.2
Zn	3.40
Mn	2.50
Cu	1.20
Mg	24.7
Ca	110.5
Na	20.30

DISCUSSION

In this work, flavonoids and phenolic compounds are increasingly recognized for their significant antioxidant properties, which play a crucial role in mitigating oxidative stress and associated health risks. The total flavonoid content of 42.5 mg/g in *Urtica dioica* aligns with findings from Albogami (2023), who identified flavonol myricetin in *U. dioica*, demonstrating its capacity to alleviate oxidative stress induced by environmental pollutants such as benzoapyrene. This

is consistent with the broader understanding that flavonoids effectively scavenge free radicals, thereby reducing oxidative damage and potentially enhancing the protective effects of *U. dioica* against aflatoxin B1 toxicity, as noted by Bhusal *et al.* (2022). The total phenolic content of 58.3 mg/g corroborates the findings of Bhusal *et al.* (2022), who compiled evidence supporting the antioxidant activity of *U. dioica*, highlighting its therapeutic potential against various diseases. In addition to flavonoids and phenolic compounds, the presence of saponins (15.6 mg/g) and tannins (12.4 mg/g) in *U. dioica* contributes to its pharmacological profile. Saponins are well-documented for their cholesterol-lowering and anti-inflammatory effects, while tannins are recognized for their astringent properties and ability to reduce oxidative stress (Bhusal *et al.,* 2022). The synergistic effects of these phytochemicals may enhance the overall therapeutic efficacy of *U. dioica*, particularly in counteracting the adverse effects of aflatoxin exposure. The research of Mhalhel *et al.* (2024) supports this notion, revealing that *U. dioica* extract can mitigate toxicity in zebrafish larvae. This multifaceted approach to health promotion underscores the importance of these bioactive compounds in *U. dioica*.

Alkaloids and steroids, although present in lower concentrations (6.8 mg/g and 3.2 mg/g, respectively), also contribute significantly to the biological activities of *U. dioica*. Alkaloids have been linked to various health benefits, including anti-cancer properties, as evidenced by studies demonstrating their potential to inhibit cancer cell proliferation (Karakol *et al.,* 2022). Similarly, steroids have been associated with anti-inflammatory effects, further contributing to protective mechanisms against aflatoxin B1-induced toxicity (Bhusal *et al.,* 2022). Devkota *et al.* reported that *U. dioica* extracts exhibit significant anti-inflammatory and antioxidant activities, which could be beneficial in managing conditions exacerbated by oxidative stress (Saponaro et al., 2020). The findings of Kasouni *et al.* (2020) on the cytotoxicity and anti-cancer activity of methanolic U. *dioica* leaf extract further emphasize its potential as a food supplement with therapeutic applications. Additionally, the work by Awoyemi et al. (2022) highlighted the pro-fertility and antioxidant activities of the ethanolic root extract of *U. dioica* in female albino rats, showcasing the plant's diverse pharmacological potential. Collectively, these findings suggest that the bioactive compounds present in *U. dioica* not only counteract the toxic effects of aflatoxins but also promote overall health and well-being.

The proximate analysis of *Urtica dioica* leaf extract revealed several key nutritional parameters that align with existing literature. The moisture content of *Urtica dioica* leaves, recorded at 9.70%, is indicative of the plant's ability to retain water, which is crucial for maintaining its physiological functions and overall health. High moisture content in plant materials can influence their shelf life and stability during storage. Other studies have reported varying moisture levels in different plant extracts, emphasizing the importance of extraction methods and environmental conditions on moisture retention (Mbagwu *et al.,* 2022).

The ash content of 3.33% reflects the mineral content of *Urtica dioica* leaves, which is essential for various physiological functions in humans. Ash content serves as an indicator of the total mineral content present in the plant material. Previous research has shown that plants with higher ash content often provide essential minerals such as calcium, magnesium, and potassium, which are vital for human health (Chaudhary *et al.,* 2023). This mineral profile can contribute to dietary recommendations, particularly in regions where mineral deficiencies are prevalent.

The crude protein content of 12.45 % in *Urtica dioica* is particularly noteworthy, as it indicates a significant source of protein for human consumption. This level of protein is comparable to other leafy vegetables and suggests that *Urtica dioica* could serve as a valuable dietary supplement, especially in vegetarian and vegan diets. Studies have shown that the protein content in plant materials can vary widely depending on the species and environmental conditions (Chaudhary *et al*., 2023). The presence of essential amino acids in the protein profile of Urtica dioica further enhances its nutritional value.

The crude fibre content of 3.00 % indicates that *Urtica dioica* leaves can contribute to dietary fibre intake, which is essential for digestive health. Dietary fibre plays a crucial role in regulating bowel movements and preventing constipation, as well as in managing blood sugar levels and cholesterol (Sambou *et al*., 2022). The fiber content in *Urtica dioica* is consistent with findings from other studies on leafy greens, which typically report similar fiber levels. The inclusion of *Urtica dioica* in the diet could, therefore, support gastrointestinal health and contribute to overall well-being.

The crude lipid content of 5.88 % suggests that *Urtica dioica* leaves contain a moderate amount of fats, which are essential for various bodily functions, including hormone production and nutrient absorption. The lipid profile of *Urtica dioica* may include beneficial fatty acids, such as omega-3 and omega-6, which are known for their anti-inflammatory properties (Haslina *et al.,* 2023). Research on other plant extracts has shown that methanol extraction can effectively isolate lipophilic compounds, enhancing the nutritional profile of the extracts (Mbagwu *et al.,* 2022; Sheikh *et al*., 2023).

The carbohydrate content of 65.66% is the highest among the proximate components analyzed, indicating that *Urtica dioica* is a rich source of carbohydrates, primarily in the form of starches and sugars. Carbohydrates are a primary energy source for the body, and their presence in *Urtica dioica* could make it a valuable addition to the diet, particularly for individuals needing energy-dense foods (Chaudhary *et al*., 2023). The high carbohydrate content aligns with findings from other studies that highlight the nutritional importance of various plant species, reinforcing the role of *Urtica dioica* as a functional food. The proximate composition of the methanol extract of *Urtica dioica* leaves reveals significant nutritional parameters that may contribute to its ameliorative potential against AFB1 toxicity. The high carbohydrate content suggests that *Urtica dioica* could serve as an energy source, while the protein content may provide essential amino acids necessary for cellular repair and regeneration, particularly following AFB1-induced damage.

The presence of minerals such as magnesium and calcium are particularly noteworthy, as they are vital for various physiological functions, including enzyme activity and cellular signaling. Magnesium, for instance, is essential for over 300 biochemical reactions in the body, including those involved in energy production and protein synthesis (Tiisekwa, 2023). The high magnesium content (24.7 %) in *Urtica dioica* may contribute to its protective effects against oxidative stress, which is often exacerbated by AFB1 exposure. Aflatoxins are known to induce oxidative stress and disrupt mitochondrial function, leading to apoptosis and organ damage (Zhang *et al.,* 2022). Therefore, the antioxidant properties associated with magnesium could help mitigate these effects.

Zinc, another essential mineral present in the extract (3.4%), plays a critical role in immune function and has been shown to counteract the immunosuppressive effects of AFB1 (Ogallo *et al*., 2023). Chronic exposure to AFB1 is linked to immune system modulation, which can lead to increased susceptibility to infections and diseases (Ahmed *et al*., 2022). The incorporation of zinc into the diet through *Urtica dioica* may enhance the immune response and provide a protective effect against the immunotoxicity of aflatoxins.

Iron (15.2%) and copper (1.2%) are also crucial for various enzymatic processes, including those involved in antioxidant defense mechanisms. Iron is a key component of hemoglobin and is vital for oxygen transport, while copper is involved in the formation of superoxide dismutase, an important antioxidant enzyme (Albogami, 2023). The presence of these minerals *in Urtica dioica* may enhance the antioxidant capacity of the extract, thereby providing additional protection against oxidative damage induced by AFB1. Research has indicated that dietary supplementation with minerals such as zinc and magnesium can significantly reduce the toxic effects of aflatoxins in animal models (Mohammed and Sarhan, 2023). This suggests that the mineral-rich profile of *Urtica dioica* could be leveraged to develop dietary interventions aimed at reducing the health risks associated with aflatoxin exposure.

The role of *Urtica dioica* in ameliorating the effects of AFB1 can also be attributed to its phytochemical constituents, which include flavonoids and polyphenolic compounds known for their antioxidant properties (Gao *et al.,* 2022). The methanol extract of *Urtica dioica* is rich in these bioactive compounds, which can scavenge free radicals and reduce oxidative stress, further supporting the protective effects against AFB1 toxicity. Studies have demonstrated that flavonoids can modulate oxidative stress and inflammation, thereby providing a synergistic effect when combined with essential minerals (Foerster *et al.,* 2022).

In addition to its mineral content, the potential of Urtica dioica to mitigate AFB1 toxicity may also be linked to its ability to enhance nutrient absorption and bioavailability. The presence of certain phytochemicals in *Urtica dioica* can facilitate the absorption of minerals in the gastrointestinal tract, promoting better overall nutritional status (Xia *et al.,* 2023). This is particularly important in aflatoxin exposure, as malnutrition can exacerbate the toxic effects of mycotoxins (Hoteit *et al.,* 2024). By improving mineral absorption, *Urtica dioica* may help to counteract the nutritional deficiencies often associated with chronic aflatoxin exposure.

Furthermore, the findings regarding the mineral composition of *Urtica dioica* can be contextualized within the broader framework of dietary strategies aimed at reducing aflatoxin-related health risks. Aflatoxins, particularly AFB1, are prevalent in various food sources, including grains and nuts, and pose a significant threat to food safety and public health (Li *et al.,* 2024). The incorporation of *Urtica dioica* into the diet could serve as a practical approach to enhance the nutritional quality of foods and provide a protective mechanism against the harmful effects of aflatoxins.

CONCLUSION

In conclusion, this study demonstrated the potential of *Urtica dioica* leaf extract as a mycotoxin combatant, primarily against mycotoxin toxicity. The methanolic extract of *U. dioica* leaves is rich in bioactive phytochemicals, particularly total phenolics and flavonoids, which exhibit strong antioxidant properties. These compounds, alongside saponins,

tannins, alkaloids, and steroids, contribute to the plant's pharmacological profile, supporting its protective role against oxidative stress and toxin-induced damage. The high carbohydrate and protein content in *U. dioica*, complemented by a diverse mineral profile—especially calcium, magnesium, zinc, and iron—underscores its nutritional and therapeutic potential. The mineral composition in *U. dioica* supports immune function, enhances antioxidant defenses, and may counteract the immunosuppressive effects of AFB1. By supplying essential minerals and phytochemicals, *U. dioica* could mitigate aflatoxin-related toxicity through enhanced antioxidant activity and improved nutrient bioavailability. This suggests that *U. dioica* leaf extract could be an effective natural supplement for managing aflatoxin exposure, offering both nutritional and health benefits. Further research on its bioactive mechanisms and practical applications in human diets may enhance its role in combating mycotoxin-induced health issues.

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A REVIEW: PREVALENCE OF MYCOTOXINS IN DRIED FISH AND FOOD SAFETY IN NIGERIA

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ABSTRACT
Mycotoxins are toxic secondary metabolites produced by molds. Which represent a growing concern in the field of food safety. This study delves into the prevalence of mycotoxins in dried fish and its far-reaching implications. The research explores the extent of mycotoxin contamination, its health and economic consequences, and recommended strategies for mitigation. Key findings indicated the presence of mycotoxins in dried fish, these pose significant health risks to consumers. The study also highlights their impact on the quality and marketability of dried fish and the challenges this presents to the food industry. Emerging trends, including the influence of climate change, the discovery of new mycotoxins, and the need for global surveillance and harmonization of regulations, further complicate the landscape of mycotoxin contamination. Small holder farming, biomarker development, and sustainable mitigation strategies stand out as ongoing challenges and research needs. Regulatory harmonization and enhanced public awareness are critical to addressing these challenges. The implications for food safety are profound, affecting trade, consumer confidence, and the cost of control measures. Compliance with mycotoxin regulations and standards is essential, while a multifaceted approach is required to tackle this complex issue. The study recommends enhanced monitoring and surveillance, public awareness and education campaigns, regulatory harmonization, support for small holder farmers, biomarker development, and sustainable mitigation strategies. Collaborative efforts among governments, the food industry, research institutions, and consumers are crucial to ensuring the safety and security of the global food supply chain. These recommendations provide a roadmap for mitigating mycotoxin contamination and enhancing food safety for consumers worldwide.

Keywords: Fish, Food safety, Fungi, Mycotoxin and Mitigation.

INTRODUCTION
Dried fish has been a staple food in diets across various cultures for centuries, valued for its long shelf life and convenience (Naylor *et al.,* 2021). However, recent concerns have arisen regarding the presence of mycotoxins in dried fish and its potential impact on food safety. Mycotoxins are toxic secondary metabolites produced by certain fungi, and their contamination in food is a global issue (Imran *et al.,* 2020). Mycotoxins are secondary metabolites (substances manufactured by plants that make them competitive in their own environment) of molds that can contaminate a wide range of agricultural products, including grains, nuts, and dried fish. These toxic compounds, including aflatoxins, ochratoxins, and fumonisins, have been linked to adverse health effects, such as liver damage, kidney problems, and even carcinogenicity (Oliveira & Vasconcelos, 2020). Consequently, understanding the extent of mycotoxin contamination in dried fish and implementing strategies to mitigate this risk is essential for ensuring food safety.

Dried fish, a staple in many traditional diets, plays a crucial role in providing a source of protein and essential nutrients in regions where fresh seafood is not readily available (Kari *et al.,* 2022). Its history traces back centuries, and it remains an integral part of the culinary heritage of numerous cultures worldwide. However, concerns have arisen regarding the safety of dried fish products due to the potential presence of mycotoxins, toxic compounds produced by molds (Fitri *et al.,* 2022). Historically, drying fish was a means of preserving this highly perishable food source, relying on sun drying or smoking methods. These traditional practices, while effective at extending the shelf life of fish, did not always address the risks of mold growth and mycotoxin contamination. Dried fish is particularly susceptible to mycotoxin contamination due to its moisture content and the conditions in which it is traditionally prepared and stored (Fitri *et al.*, 2022). In regions with high humidity and inadequate drying facilities, dried fish could become a breeding ground for molds, increasing the likelihood of mycotoxin production (Abdelmotilib *et al.,* 2021). Additionally, the lack of standardized monitoring and testing practices can contribute to mycotoxin contamination going undetected.

As global trade continues to expand, dried fish products are increasingly crossing borders, necessitating rigorous food safety measures to protect consumers (Barrett, 2021). Understanding the prevalence of mycotoxins in dried fish, along with the factors contributing to their presence, is crucial for addressing this emerging food safety challenge. The prevalence of mycotoxins in dried fish presents a multifaceted challenge to both food safety and the preservation of cultural dietary traditions. Despite the significant role dried fish plays in the diets of communities around the world, there exists a lack of comprehensive research and data on the extent and nature of mycotoxin contamination in dried fish products. The consumption of dried fish is deeply ingrained in the culinary traditions of many cultures, serving as a valuable source of protein and essential nutrients. However, this cherished tradition is increasingly marred by the potential presence of mycotoxins, toxic compounds produced by molds, in dried fish products.

Mycotoxins pose a significant threat to public health when present in food products. They are known to cause various health problems, including liver and kidney damage, immune system suppression, and cancer. Investigating the prevalence of mycotoxins in dried fish is crucial, as this widely consumed food item can potentially expose a large number of people to these health risks. Dried fish production is a vital economic activity in many regions, supporting livelihoods and contributing to local economies (Berenji *et al.*, 2021). Ensuring the safety of dried fish products is essential to maintain consumer confidence and protect the economic interests of those involved in the industry.

The significance of this study lies in its potential to shed light on the prevalence and levels of mycotoxin contamination in dried fish products. Understanding the extent of this issue is crucial due to the harmful health effects associated with mycotoxin ingestion, ranging from acute toxicity to chronic health problems (Frisvad *et al.,* 2019). Furthermore, the presence of mycotoxins in dried fish can have economic implications, affecting trade and consumer confidence in these products (FAO, 2018).

Mycotoxins are toxic secondary metabolites produced by various species of molds (fungi) that can contaminate a wide range of agricultural products, including grains, nuts, and dried fish (Cinar & Onbaşı, 2019). These compounds pose a significant threat to human and animal health due to their potential to cause a variety of adverse effects, including acute and chronic illnesses. Several types of mycotoxins have been identified, each produced by different fungal species. Some of the most common mycotoxins include: Aflatoxins, Ochratoxins, Fumonisins, Zearalenone, Deoxynivalenol (DON), and Patulin, T-2 Toxin. Mycotoxins are often associated with mold growth in agricultural commodities, especially when conditions favor fungal proliferation. Several factors contribute to mycotoxin contamination in food: Environmental Factors, Storage and Handling, Crop Conditions, Fungal Species.

Overview of Dried Fish

Dried fish is a traditional method of preserving fish that has been practiced for centuries in various cultures around the world (Akintola & Fakoya, 2017). This preservation technique involves removing moisture from fresh fish through natural processes like sun drying or smoking. The resulting dried fish products are known for their extended shelf life, concentrated flavors, and nutritional benefits.

Production Process

Preparation, Salting, Drying, Packaging.

Types of Dried Fish

Sun-dried fish, smoked dried fish, and salted dried fish.

Nutritional Benefits

Dried fish retains much of its nutritional value, including protein, vitamins, and minerals, making it a nutritious food source. It is often rich in omega-3 fatty acids, which have numerous health benefits (Byrd *et al.,* 2021).

Challenges and Food Safety

Despite its advantages, the drying process can sometimes be susceptible to contamination by molds and mycotoxins, which pose food safety risks. Proper drying techniques, storage conditions, and quality control measures are essential to minimize these risks.

Global Consumption Trends of Dried Fish

Dried fish is consumed globally, and its popularity varies across regions due to cultural preferences, availability of seafood resources, and dietary traditions. Understanding global consumption trends can provide insights into the importance of dried fish in different parts of the world; Asia-Pacific Region, Africa, Europe and North America and South America.

Importance of Dried Fish in Diets

Dried fish holds significant importance in diets worldwide, offering a range of nutritional and practical benefits. Dried fish is a rich source of protein, making it a valuable dietary component, especially in regions where access to fresh meat or seafood is limited (Rasul et al., 2021). The protein content in dried fish helps meet daily protein requirements, contributing to overall health and growth. Drying preserves essential nutrients, including vitamins and minerals, making dried fish a nutritious option. Retaining these nutrients ensures that consumers receive a valuable source of vitamins and minerals, such as vitamin B12 and selenium (Kipper & Sulg, 2016).

Dried fish has an extended shelf life due to its low moisture content, making it an excellent choice for long-term storage (Kanatt, *et al.,* 2020). This long shelf life is especially advantageous in areas with unreliable refrigeration or limited access to fresh foods. Dried fish is lightweight and easy to transport, making it suitable for communities with nomadic lifestyles or limited access to markets (Kruijssen *et al.,* 2020). Its portability allows for consumption in various settings, including remote or outdoor locations. Dried fish often holds cultural significance in many culinary traditions. It is used to add unique flavors to dishes and preserve cultural heritage through traditional recipes (Gopakumar & Arunlal, 2016). Dried fish can be used in various culinary applications, from soups and stews to stir-fries and curries. Its versatility in cooking allows for the creation of a wide range of flavorful and satisfying dishes (Morgan, *et al.,* 2006).

In some regions, dried fish production supports sustainable fishing practices and the livelihoods of coastal communities. It can contribute to the conservation of fishery resources when managed responsibly (Su *et al.,* 2020).

Factors Contributing to Contamination of Dried Fish

Contamination of dried fish can occur at various stages of production, processing, and storage. Understanding the factors contributing to this contamination is essential for ensuring food safety. Poor hygiene and sanitation practices during fish handling, drying, and storage can introduce contaminants. Inadequate cleaning of drying racks, equipment, and processing areas can lead to microbial contamination (Mwasulama *et al.,* 2021). Environmental factors such as high humidity and warm temperatures can promote mold growth on drying fish. Unprotected drying under direct sunlight without proper shelter can also lead to microbial contamination (FAO, 2013). Incomplete drying of fish can leave moisture content higher than desired, creating conditions for microbial growth and spoilage. Properly drying fish to the required moisture level is crucial for preventing contamination (Wagacha & Muthomi, 2008). Improper storage conditions, such as exposure to moisture, heat, or pests, can compromise the quality and safety of dried fish. Adequate packaging and storage facilities are essential to prevent contamination during storage (Marriott *et al.,* 2018).

Insects, including flies and beetles, can lay eggs on drying fish, leading to larval infestation. Infested fish can harbor harmful microorganisms introduced by insects (Barkai-Golan, 2008). Cross-contamination can occur when dried fish comes into contact with contaminated surfaces or equipment (Carrasco *et al.,* 2012). Separation of processing and drying areas from areas with potential contaminants is essential. The presence of molds (mycoflora) on drying fish can lead to mycotoxin contamination (Adebayo-Tayo *et al.,* 2008). Mycotoxins, such as aflatoxins, can be produced by molds and pose health risks. The choice of drying methods, including sun drying and smoking, can impact the safety of dried fish (Belton *et al.,* 2022). Proper smoking practices, such as using non-contaminated smoke sources, are crucial.

Diseases in Dried Fish

Scombroid Poisoning, Botulism, Anisakiasis, Bacterial Infections, Histamine Poisoning.

Mitigation and Prevention Strategies

Good Manufacturing Practices (GMP) for Dried Fish, Good Manufacturing Practices (GMP) are a set of guidelines and principles that ensure the production of safe and high-quality food products (Mtewa *et al.,* 2020). These practices are essential in the dried fish industry to prevent contamination, maintain product integrity, and protect the health of consumers.

Proper hygiene and sanitation practices are fundamental in preventing contamination. Workers should follow strict hygiene rules, and facilities and equipment should be regularly cleaned and disinfected (FAO, 2013).

Employees involved in dried fish processing should receive training on GMP principles, including proper handling, storage, and hygiene practices. Well-trained personnel are more likely to follow safe practices (Okpala & Korzeniowska, 2023). Facilities for drying and processing fish should be designed to minimize environmental contamination. Adequate airflow and protection from pests are important considerations (Coronel *et al.,* 2022). Quality control measures should be in place to identify and reject contaminated or substandard fish. Regular inspections can help ensure that dried fish

products meet quality and safety standards (Hoque & Myrland, 2022). Drying and storage facilities should maintain appropriate temperature, humidity, and airflow conditions to prevent mycotoxin growth and spoilage. Dried fish should be stored in moisture-proof packaging to maintain quality (FAO, 2013).

Effective pest control programs should be implemented to prevent infestations that can contaminate the product. This includes regular monitoring and the use of safe pest control methods (Hervet & Morrison III, 2021). Hazard Analysis and Critical Control Points (HACCP) Stages include; Hazard Identification, Critical Control Points (CCPs), Establishing Critical Limits, Corrective Actions, Documentation and Record Keeping, Verification and Validation

Storage and Handling Best Practices for Dried Fish

Proper storage and handling of dried fish are crucial to maintain its quality, prevent spoilage, and ensure food safety. Here are some key guidelines for the storage and handling of dried fish: Dried fish should be stored in a cool, dry, and well-ventilated area. Proper storage conditions help prevent moisture absorption, which can lead to mold growth and mycotoxin production (FAO, 2013).

Use airtight containers or vacuum-sealed bags to protect dried fish from air and moisture, which can cause rancidity and loss of quality (FAO, 2013). Protect dried fish from pests by storing it in pest-resistant containers and maintaining a clean storage environment (FAO, 2013). Dried fish should be protected from direct sunlight, as UV radiation can deteriorate the quality of the product (Chiozzi *et al.,* 2022). Label containers with the date of drying and storage to monitor freshness and safety (FAO, 2013). Maintain an optimal relative humidity level (usually below 65%) to prevent mold and mycotoxin formation (Viviane *et al.,* 2021).

Wash hands thoroughly with soap and water before handling dried fish to prevent contamination (Sobuj *et al*., 2022). Ensure that utensils and containers used for dried fish are clean and sanitized to prevent bacterial growth (Viji *et al*., 2022). Implement pest control measures in storage areas to avoid infestations and protect the product from contamination (FAO, 2013). Periodically inspect stored dried fish for signs of mold, moisture, or pests, and remove any affected products (Jeyakumari, 2022). Store dried fish away from strong odors and contaminants that can affect its flavor (Zhang *et al.,* 2021).

Implications for Food Safety

Mycotoxin contamination has significant implications for food safety: Mycotoxins are toxic compounds that can cause acute and chronic health issues when ingested. These health risks range from gastrointestinal problems to more severe conditions, including liver and kidney damage, immunosuppression, and even cancer (Chadban *et al.,* 2020).

Mycotoxin contamination can result in significant economic losses. It affects the market value of crops, leads to food waste, and necessitates costly control and mitigation measures (Kagwathi *et al.,* 2023). Mycotoxin-contaminated food products can erode consumer confidence in the safety and quality of the food supply. This can have long-lasting effects on food industries and the reputation of brands (Gbashi *et al.,* 2021).

Stringent mycotoxin regulations in international trade can create trade barriers. Compliance with maximum permissible mycotoxin levels is necessary to access global markets (Mukhtar *et al.,* 2023). Mycotoxin contamination can undermine food security by reducing the availability and safety of staple foods, particularly in regions heavily reliant on crops vulnerable to mycotoxin production (Godde *et al.,* 2021).

Preventive Measures

Food safety systems must incorporate preventive measures to control mycotoxin contamination. This includes effective agricultural practices, post-harvest handling, storage, and processing techniques (Nada *et al.,* 2022). Consumer and industry education is crucial to raise awareness about mycotoxin risks. Proper food handling, storage, and preparation are essential to reduce exposure (Jeebhay *et al.,* 2019).

CONCLUSION

The study on the prevalence of mycotoxins in dried fish and its implications for food safety has yielded several important findings and insights. This research has shed light on the following key points: The study has confirmed that dried fish products are susceptible to mycotoxin contamination, a concern that is of increasing significance in the context of global food safety. The presence of mycotoxins in dried fish underscores the potential health risks associated with the consumption of contaminated products. These risks include both acute and chronic health issues, making the need for mycotoxin control paramount.

The research has identified various factors contributing to mycotoxin contamination in dried fish, including storage conditions, climate, and handling practices. Understanding these factors is crucial in developing effective mitigation strategies. The maximum levels for mycotoxins in food are very low due to their severe toxicity. For example, the maximum levels for aflatoxins set by the Codex in various nuts, grains, dried figs and milk are in the range of 0.5 to 15 μg/kg (a μg is one billionth of a kilogram).

RECOMMENDATIONS

Based on the findings and conclusions of a study on the prevalence of mycotoxins in dried fish and their implications for food safety, the following recommendations can be made: Establish comprehensive monitoring and surveillance systems to regularly assess mycotoxin levels in dried fish. This includes both routine testing of products in the market and field surveys to identify contamination hotspots. Invest in further research to better understand the factors influencing mycotoxin contamination in dried fish. This research should also focus on the development of innovative detection methods, mitigation strategies, and sustainable approaches.

Promote education and awareness among producers, processors, and consumers regarding the risks of mycotoxin contamination and the best practices for prevention and control. Work towards harmonizing mycotoxin regulations and standards across countries and regions to facilitate international trade while ensuring food safety. This can involve the alignment of acceptable mycotoxin levels and maximum residue limits.

Promote the adoption of Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) in the production, processing, and storage of dried fish. These practices can significantly reduce the risk of mycotoxin contamination. Continue research on biomarkers for mycotoxin exposure in humans and animals. Developing reliable biomarkers can aid in early risk assessment and intervention. Investigate and implement sustainable methods for mycotoxin control in dried fish production, including the use of biocontrol agents, mycotoxin binders, and genetic resistance.

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DEVELOPMENT OF CORNCOB-BASED BIOCHAR-CELLULOSIC MATRIX FOR HEAVY METAL REMEDIATION FROM WASTEWATER

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ABSTRACT
Freshwater contamination by heavy metals poses significant threats to human health and the ecosystem. This research explores the use of corncob-based biochar-cellulosic matrix for remediation of heavy metals from wastewater. Biochar-cellulosic composites were prepared in varying ratios (0:1, 1:2, 2:3, and 1:1) of biochar to cellulose. The physicochemical properties of wastewater and heavy metal (lead, copper, zinc, chromium and cadmium) quantification were determined using standard procedures. Acid hydrolysis and alkaline treatment was adopted for cellulose extraction while the biochar was produced through pyrolysis of corncob. The functional groups of the matrix were characterized using FTIR spectroscopy. The result showed that extraction of cellulose from corncob had a yielded of 33.65 %. Treatment of wastewater with the matrix resulted in significant improvements in water quality parameters, with reduction in turbidity from 423.0 NTU to 78.0 NTU, total dissolved solid (TDS) decreased from 60.6 to 35.5 mg/l, conductivity reduced from 49.2 to 39.3 nS/cm, while maintaining pH levels between 7.6-8.3 and temperature ranged between 28.0-29.5 °C. Wastewater treatment with the matrix also demonstrated significant reduction in heavy metal concentration. Lead decreased by 94.74 %, copper concentration was reduced by 58.33 %, zinc concentration decreased by 48.11-64.15 %. Furthermore, chromium concentration in wastewater reduced by 77.78 % while 42.86-85.71 % reduction was observed for cadmium. These findings demonstrated that corncob-based biochar-cellulosic matrix, particularly at a 1:1 ratio, offers a promising, sustainable solution for wastewater remediation, effectively removing heavy metals while improving overall water quality parameters.

Keywords: Cellulose, Biochar, Heavy metals, Wastewater, Corncob

INTRODUCTION
The increasing demand for sustainable and efficient wastewater treatment methods has led to the exploration of various materials and technologies. Among these, biochar has gained significant attention due to its potential for environmental remediation. Biochar, a carbon-rich material derived from the pyrolysis of organic matter, has been widely studied for its ability to adsorb contaminants from water (Lehmann and Joseph, 2015). Corncob, an agricultural waste product, is an abundant and renewable resource that can be converted into biochar through pyrolysis. The use of corncob for biochar production not only provides a sustainable waste management solution but also enhances the economic value of agricultural residues (Rattanaphaiboon *et al*., 2022). The effectiveness of biochar in wastewater treatment is largely dependent on its surface area, porosity, and functional groups. For instance, biochar produced at 500°C has shown high adsorption capacities for lead (Pb) and chromium (Cr), making it a promising material for heavy metal remediation (Lee *et al*., 2013). These properties can be tailored by optimizing the pyrolysis conditions, such as temperature and residence time (Drané *et al*., 2024). Higher pyrolysis temperatures generally increase the surface area and porosity of biochar, enhancing its adsorption capacity (Shakya and Agarwal, 2023). Incorporation of cellulosic materials into the biochar matrix can further improve its structural integrity and adsorption efficiency (Divyangkumar *et al*., 2024). Moreover, the modification of biochar with cellulosic materials can enhance its mechanical strength and stability, making it more effective in dynamic water treatment systems (Basu, 2018). This study therefore aimed at developing a matrix with enhanced adsorption properties for wastewater remediation. This study highlights the potential of corncob-based biochar-cellulosic matrices as a viable solution for wastewater remediation, paving the way for future advancements in this field.

MATERIALS AND METHODS

Biochar Production

The corn cobs were cleaned to remove dirt, debris, and foreign materials. They were then dried in an oven at 60°C for 24 hours. The corn cobs were then loaded into a pyrolysis reactor. The pyrolysis temperature was then set to 500 °C and left to run for 2 hours. The pyrolysis reactor was then left to cool and the biochar removed from the reactor. The biochar was then pulverized and stored in an airtight bag to prevent moisture absorption.

Cellulose Extraction from Corn Cob

The cellulose extraction was carried out at the Department of Biochemistry laboratory, Federal University of Technology, Minna. Corn cobs were cleaned, dried, and ground into a fine powder which was then subjected to acid hydrolysis using dilute sulfuric acid (H_2SO_4) at a concentration of 2 % (v/v) with a solid-to-liquid ratio of 1:10 (w/v) at 120 °C for 60 minutes in an autoclave. The resulting slurry was then cooled and filtered. The solid residue was washed thoroughly with distilled water and its pH level measured using a pH meter until neutral pH was achieved. The acid-treated material was then subjected to bleaching using 4 % NaOH at 70°C for 2 hours with a solid-to-liquid ratio of 1:10 (w/v). The bleached pulp was again washed with distilled water until neutrality and oven dried at 60°C for 24 hours.

Cellulose yield calculation

The yield of cellulose is calculated as the ratio of the dry weight of extracted cellulose to the initial dry weight of corn cob powder (Sharma *et al.*, 2020). The extracted cellulose is then used for further experiments in the production of biochar cellulosic matrix for heavy metal remediation in wastewater.

$$Cellulose\ yield = \frac{dry\ weight\ of\ extracted\ cellulose}{initial\ dry\ weigh\ \ of\ corn\ cob\ powder} \times 100 \quad \ldots\ldots\ldots\ldots 1$$

Biochar-cellulose matrix preparation

Preparation of cellulose solution

5 g of microcrystalline cellulose was weighed using an analytical balance. In a 250 mL beaker,100 mL of distilled water was measured. The weighed cellulose was added gradually to the distilled water while stirring with a magnetic stirrer at 600 rpm. Stirring was done for 2 hours at room temperature to ensure complete dissolution.

Incorporation of biochar into cellulose matrix

A series of biochar-cellulose mixtures with ratio 1:0, 1:2, 2:3 and 1:1 was prepared by weighing 2.5g, 3.33g and 5g of biochar powder was added to the cellulose solution in small increments (approximately 1 g at a time). Using a magnetic stirrer at 5000 rpm for 5 minutes after each addition to ensure even distribution. Continue this process until all the biochar is incorporated.

Casting the matrix

20 mL of the biochar-cellulose mixture was measured using a graduated cylinder. The measured mixture was poured into a glass Petri dish, a stainless-steel spreading tool was used to distribute the mixture uniformly across the dish. The Petri dishes were placed in a water bath at 60°C (±1°C). The samples were left to dry for until constant weight was achieved. The dried matrices were allowed to cool at room temperature.

Water absorbing capacity

The biochar-cellulosic matrix samples were cut into 2 cm × 2 cm pieces and dried in an oven at 105°C for 24 hours. After cooling in a desiccator for 30 minutes, the initial weight (W_0) of each sample were recorded using an analytical balance. The samples were immersed in distilled water at room temperature (23 ± 2°C) for 24 hours. After immersion, samples were removed and gently blotted with filter paper to remove surface water. The final weight (W_t) was recorded immediately.

The water absorbing capacity was calculated using the equation.

$$WAC = \frac{Weigh\ \ of\ Hydrated\ Residue - Ini\ \ \ \ Dry\ Weig}{Initial\ Dry\ Weigh} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots 2$$

Solubility test

A 1 g sample of the matrix was weighed and placed in a glass beaker containing 100 mL of distilled water at room temperature (25±2°C). The mixture was gently stirred at 200 rpm for 30 minutes using a magnetic stirrer. After stirring, the solution was filtered through Whatman No. 1 filter paper, and the undissolved material was collected and dried in an oven at 105°C for 3 hours until constant weight was achieved.

The solubility percentage was calculated using the equation

$$Solubility\ (\%) = \frac{Initial\ Weight - Final\ Weight\ of\ Insoluble\ Fraction}{Initial\ Weigh} \times 100 \quad \ldots\ldots\ldots\ldots 3$$

Moisture content

The moisture content of the biochar-cellulosic matrix samples was determined using the oven-drying method. Approximately 2 g of each sample was weighed accurately in pre-dried aluminum dishes. The samples were then placed in a convection oven at 105°C for 24 hours to ensure complete moisture removal. After drying, the samples were cooled in a desiccator to room temperature and reweighed. The moisture content was calculated as the percentage of weight loss relative to the initial sample weight. This process was repeated in triplicate for each sample to ensure accuracy and reproducibility of results. The average moisture content was then calculated and reported for each biochar-cellulosic matrix composition.

$$Moisture\ content\ (\%) = \frac{(W2 - W3)}{(W2 - W1)} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 4$$

Where; W1= Weight of the empty container

W2= Weight of the container with the wet sample

W3= Weight of the container with the dried sample

Treatment of Wastewater with Biochar-cellulosic Matrix

Wastewater which was gotten from was treated with the varying ratio (1:0, 1:2, 2:3 and 1:1) of biochar cellulosic matrix. 1g of each sample was weighed and added into 200ml of wastewater sample, then left to stand for 2 hours, after which the wastewater samples were decanted.

Heavy Metal Analysis

Heavy metals analysis was carried out on the wastewater samples which were treated with the developed biochar-cellulosic composite. The wastewater samples were digested by adding 5ml of 99% concentrated HCl to 50 ml of each wastewater sample and placed on a hot plate and left to vaporize to give 25 ml. This step was repeated for all samples including the blank sample. The wastewater samples were then filtered using a filter paper and funnel into a wash bottle. The samples were then aspirated into a flame atomic absorption spectrometer, where absorbance was measured and compared against known metals concentration.

Water quality parameters analysis

Water quality parameters were measured using standard analytical methods. The pH was determined using a calibrated digital pH meter (Model XYZ) with automatic temperature compensation. Total Dissolved Solids (TDS) and electrical conductivity were measured using a multiparameter meter (Model ABC), which was calibrated using standard KCl solution prior to measurements. Temperature readings were taken simultaneously using the temperature sensor integrated within the multiparameter meter. Turbidity was measured using a calibrated turbidity meter (Model DEF) and expressed in Nephelometric Turbidity Units (NTU). The probes were rinsed with distilled water and dried between measurements to prevent cross-contamination.

Fourier-transformed infrared spectroscopy (FT-IR)

The developed matrices were prepared for FT-IR by crushing them into powder which were then embedded in KBr pellets and analysed using Agilent 620 FTIR spectrophotometer. The spectra were recorded in the absorption band range of 4000 – 400cm-1 $^{-1}$, with a spectral resolution of 4 cm^{-1}.

RESULT AND DISCUSSION

Cellulose yield

The cellulose weight gotten after extraction was 84.13 g from 250 g of corn cob sample. The cellulosic yield from corncob was 33.65%

FT-IR result

The FT-IR result is presented in Table 1. The FT-IR spectra for four samples Sample A, Sample B, Sample C and Sample D were obtained and analyzed for characteristic absorption bands. The key peaks and their assignments are summarized in Table 1.

The FT-IR spectra of all four cellulose samples revealed common absorption bands corresponding to key functional groups in cellulose. The presence of strong absorption peaks at 849 cm^{-1} and 1103 cm^{-1} across all samples indicates that the β-(1→4)-glycosidic linkages are preserved, confirming the cellulose structure. The broad absorption band

around 3280 cm^{-1}, associated with O-H stretching vibrations, suggests that all samples have extensive hydrogen bonding, characteristic of cellulose's hydroxyl groups. This also reflects the hydrophilic nature of cellulose.

However, notable differences were observed between the samples. The 1423 cm^{-1} peak, indicative of crystalline cellulose, was more intense in Sample A (67.95) compared to Sample D (65.45) and Sample C (62.95), suggesting that Sample A has a higher degree of crystallinity. This could imply that Sample A has undergone less processing or is more structurally ordered than the other two samples.

Additionally, Sample B, C and D exhibited a distinct peak at 2117 cm^{-1}, which was absent in Sample A. This peak could correspond to the presence of impurities or chemical modifications, such as carboxyl groups (C≡O), possibly introduced during the extraction or processing of the cellulose. The high intensity of this peak in Sample B (90.58), Sample C (92.03) and Sample D (91.31) suggests that these samples may have been subjected to similar treatment processes or contain similar impurities. The O-H bending peak at 1640 cm^{-1}, which indicates absorbed water, was slightly higher in Sample A (77.86) compared to Sample B (70.63), Sample C (72.52) and Sample D (73.67). This suggests that Sample A may have a higher moisture content, which could be due to differences in storage conditions or sample handling. Overall, the FTIR analysis confirms that all four samples are primarily composed of cellulose, but varying crystallinity, moisture content, and potential impurities distinguish them.

Table 1: FT-IR result for developed biochar-cellulosic composite

Wavenumber (cm^{-1})	Sample A (0:1) intensity	Sample B (1:1) intensity	Sample C (1:2) intensity	Sample D (2:3) intensity	Functional Group Assignment
849	62.64	54.29	56.15	57.69	C-O-C stretching β-glycosidic linkages
1028	20.33	22.15	21.35	21.28	C-O stretching (Carbohydrates)
1103	46.37	45.68	45.44	45.83	C-O-C stretching β-glycosidic linkages
1319	64.55	59.20	60.07	61.27	CH_2 bending (Crystallinity)
1423	67.95	-	62.95	65.45	CH2 bending (Crystallinity)
1640	77.86	70.63	72.52	73.67	O-H bending (Water)
2117	-	90.58	92.03	91.31	Biochar functional group
2884	72.22	67.46	68.16	69.28	C-H stretching (Aliphatic)
3280	42.70	42.34	42.73	42.59	O-H stretching (Hydrogen bonding)

Functional properties of the developed corncob-based biochar matrices

Table 2 shows the water absorbing capacity (WAC), solubility, moisture content and swelling capacity of the developed matrices. The water absorbing capacity of the 1:1 matrix proved to be the highest with a value of 300 % with the 0:1 matrix having a value of 232 %. The solubility of the developed matrix was highest in the 2:3 matrix with a value of 0.79 %, moisture content of the 1:2 matrix was the highest with 9.9 % being the highest value among the developed matrices. The swelling capacity of the 1:2 matrix was also the highest with a value of 24.33%.

Table 2: Physical Properties of the Developed Matrices

Parameters	A (0:1)	B (1:2)	C (2:3)	D (1:1)
WAC (%)	232	255	270	300
Solubility (%)	0.57	0.63	0.79	0.73
Moisture Content (%)	9.1	9.9	9.6	9.7
Swelling capacity (%)	22	24.33	18.33	16

Physicochemical result of treated wastewater

Table 2 shows the tested physicochemical result of the wastewater before and after treatment with the developed biochar-cellulose matrix. The pH result for all the water samples fell between the accepted range for NSDWQ standard. Turbidity for all the samples were above the NSDWQ standard, however, the biochar-cellulose matrix showed high reduction from the untreated wastewater with the 1:1 ratio having a value of 78.0 NTU compared to the untreated water with 423.0 NTU. Total dissolved solids (TDS) in the wastewater samples were highest in the untreated water sample

with value of 60.6 mg/L and the 1:1 sample having a value of 35.5 mg/L. Conductivity for the untreated water sample measured at 49.2 nS/cm while the 1:1 sample had the least value at 39.3 nS/cm.

Table 3: Physicochemical Properties of the Corncob-based Biochar-cellulosic Matrix Treated Wastewater

Samples	pH	Temperature (°C)	Turbidity (NTU)	TDS (mg/l)	Conductivity (nS/cm)
Raw Water	7.7	28.0	423.0	60.6	49.2
A (0:1)	8.3	29.1	356.0	53.2	44.6
B (1:2)	8.3	29.5	138.0	45.9	42.4
C (2:3)	7.6	29.4	105.0	40.6	40.7
D (1:1)	8.1	29.4	78.0	35.5	39.3
NSDWQ	6.5-8.5	Ambient	5.0	500	1000

NSDWQ: Nigerian Standard for Drinking Water Quality

Heavy metals adsorption

The results in Table 4 shows the percentage adsorption of heavy metals by the developed matrices. For zinc, the 0:1 matrix proved to be more effective removing 64.15% of zinc from the wastewater, 2:3 matrix effectively removed 85.71% of cadmium significantly higher than other matrices, 58.33% of copper was removed by both the 1:2 and 1:1 matrix as they proved the most effective for copper removal from wastewater, lead adsorption by the 1:1 matrix was seen to be highly effective at 94.74% while chromium was significantly adsorbed by the 1:2 matrix.

Table 4: Heavy Metals Adsorption by the Developed Matrices

Sample	Zinc (%)	Cadmium (%)	Copper (%)	Lead (%)	Chromium (%)
A (0:1)	64.15	57.14	33.33	42.11	40.74
B (1:2)	51.89	57.14	58.33	84.21	77.78
C (2:3)	63.21	85.71	33.33	36.84	72.22
D (1:1)	48.11	57.14	58.33	94.74	75.93

DISCUSSION

The need for effective wastewater treatment solutions is critical, given increasing environmental concerns about heavy metal pollution and water contamination (Bhatnagar & Sillanpää, 2017). Corncob-based biochar combined with cellulosic matrices represents a promising approach to address these issues. Biochar, a carbon-rich material obtained through pyrolysis of biomass, is particularly effective in adsorbing pollutants due to its high surface area and porous structure (Ahmad et al., 2014). Additionally, cellulose provides a hydrophilic, renewable matrix that enhances biochar's structural stability and adsorption potential, making it an ideal candidate for wastewater treatment applications.

In the study provided, corncob-based biochar was synthesized and incorporated into a cellulosic matrix to create a composite material for wastewater treatment. This biochar-cellulosic matrix was then assessed for its ability to reduce various physicochemical parameters such as turbidity, total dissolved solids (TDS), and conductivity in wastewater, as well as to remove heavy metals like zinc, cadmium, copper, lead, and chromium. Results indicated that the composite's ratio of biochar to cellulose significantly impacted its efficacy in pollutant removal.

For instance, the sample with a 1:1 biochar-cellulose ratio showed the highest reduction in turbidity (78.0 NTU) and TDS (35.5 mg/L), as compared to untreated water, which had values of 423.0 NTU and 60.6 mg/L, respectively. This decrease in turbidity and TDS suggests that biochar-cellulosic matrices are effective at capturing suspended solids and dissolved contaminants, aligning with findings by Aveling (2020), who observed a similar reduction in water turbidity when using biochar composites.

In terms of heavy metal adsorption, different matrix compositions exhibited varying levels of adsorption efficiency. The 2:3 biochar-cellulose matrix achieved the highest cadmium removal at 85.71%, while the 1:1 matrix was particularly

effective at adsorbing lead, with a removal efficiency of 94.74%. This pattern of selective heavy metal adsorption reflects the findings of Enaime et al. (2020), who noted that the adsorption potential of biochar can vary depending on the type of heavy metal and biochar composition.

The adsorption capabilities of biochar-cellulosic matrices can be attributed to several mechanisms. Firstly, the presence of carboxyl and hydroxyl groups on the biochar surface facilitates ion exchange with metal ions in wastewater, enhancing adsorption (Li et al., 2018). The high porosity of biochar further allows for physical adsorption, as contaminants are retained in the biochar's micropores. This aligns with observations by Lyu et al. (2020), who found that biochar's microporous structure plays a crucial role in capturing and retaining pollutants.

Furthermore, Fourier-transform infrared spectroscopy (FT-IR) analysis revealed the functional groups present in the biochar-cellulosic composite. Peaks associated with β-(1→4)-glycosidic linkages and O-H stretching vibrations confirm the preservation of cellulose's structure and the presence of hydrogen bonding, indicating a stable and hydrophilic matrix. The 2117 cm^{-1} peak observed in some samples suggests chemical modifications or impurities, possibly enhancing the composite's reactivity and adsorption potential (Tran et al., 2021).

The functional properties of the developed matrices, such as water absorbing capacity, solubility, moisture content, and swelling capacity, are crucial for their performance in wastewater treatment. The high-water absorbing capacity of the 1:1 matrix (300%) and the high moisture content of the 1:2 matrix (9.9%) suggest that these matrices are highly effective in retaining water and adsorbing contaminants. This is in line with the observations of Aljeboree et al. (2021), who noted that biochar's physicochemical properties enhance its adsorption capabilities.

Compared to traditional adsorbents such as activated carbon, corncob-based biochar-cellulosic matrices offer a more sustainable and cost-effective solution. While activated carbon is highly effective in pollutant adsorption, its production is energy-intensive, and it can be expensive (Yuan et al., 2018). In contrast, corncob-based biochar utilizes agricultural waste, making it an environmentally friendly alternative. Furthermore, the addition of cellulose enhances the mechanical stability and reusability of biochar, potentially lowering treatment costs.

The performance of corncob biochar-cellulosic composites also aligns with other studies on agricultural waste biochars, such as rice husk or bamboo, which have shown promising results in heavy metal adsorption. However, corncob-based biochar-cellulosic matrices exhibit unique properties that make them particularly suitable for industrial wastewater treatment applications due to their superior adsorption capacity for specific metals and ease of modification (Duan et al., 2020).

CONCLUSION

Corncob-based biochar-cellulosic matrix demonstrate substantial potential as a sustainable solution for wastewater treatment. They effectively reduce physicochemical contaminants and adsorb heavy metals, offering a viable alternative to more costly and environmentally taxing treatment methods. This approach could significantly improve water quality and contribute to sustainable wastewater management.

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PRELIMINARY PROBE INTO THE POTENCY OF *SALMONELLA AUREUS* SPECIES IN THE MITIGATION OF AFLATOXINS AND OCHRATOXINS CONTAMINATION

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ABSTRACT
The potency of *Salmonella aureus* species of bacteria in the mitigation of two mycotoxins (Aflatoxins and Ochratoxins) was investigated in this study. Aflatoxins and ochratoxins are a group of secondary metabolites produced in food and feed by two genera: *Penicillium* and *Aspergillus*. The fungal spores were cultured on potatoes dextrose agar (PDA) and the colonies so formed were sub cultured on yeast extract agar after which the matured fungal mycelium along with the media was harvested and subjected to extraction using dichloromethane (DCM) to obtain the mycotoxins (aflatoxins and ochratoxins) as secondary metabolite. The sample extract was subjected to Thin Layer Chromatography (TLC) and the plate (chromatograph) was viewed under Ultraviolet source in a UV-hood. The isolated non-pathological strain of gram-positive *S. aureus* specie collected from rocky area was tested for its aflatoxin and ochratoxin degradation capabilities. The strain was cultured on a yeast extract agar media separately contaminated with aflatoxins and ochratoxins obtained from the respective toxigenic fungal strains. Extract from these cultures, after two weeks incubation was subjected to thin layer chromatography for qualitative assessment for the presence or otherwise of the two toxins under consideration. While no fluorescence/emission was observed in the case of extract from aflatoxin contaminated dishes, very faint fluorescence was recorded from the ochratoxin contaminated dishes. The study demonstrated that non-pathological strain of gram-positive *S. aureus* species has strong and partial potency to biodegrade aflatoxin and ochratoxins respectively.

Keywords: Mycotoxins, Mitigation, *Salmonella aureus,* TLC

INTRODUCTION
Fungus is a member of a large group of eukaryotic organisms that includes microorganisms such as yeasts and moulds, as well as the more familiar mushrooms. They produce secondary metabolites called mycotoxins. Mycotoxins are a structurally dissimilar group of fungal natural products that are harmful to vertebrate animals or human when they are contaminants of feeds or food (Albera., 2020).

Aflatoxin (AF) is a highly toxic secondary metabolite that contaminates a number of crops causing a great economic loss (Afzal *et al*., 2023, CAST 2002). *Aspergillus flavus* and *A. parasiticus* has been identified as the main producers of AFs, however, several other molds and fungal species such as: *A. nomius*, *A. pseudotamarii* and *A. bombycis* also produce the toxin (Pickova *et al*.,2021). Aflatoxin is produced in certain food and animal feeds of which aflatoxin B1 (AFB1) constitutes the most harmful type of aflatoxins and is a potent hepato-carcinogenic, mutagenic, teratogenic and also suppresses the immune system (Oyesigye *et al*., 2021). $AFB_1$was classified as group I carcinogen by the International Agency for Research on cancer (Loomis, 2018).

Ochratoxins (OT) are a group of secondary metabolites produced by two genera: *Penicillium* and *Aspergillus* (Atumo, 2020). OT is a polyketide-derived secondary metabolite which contains a dihydrocoumarin moiety coupled to al-β-phenylalanine (Phe), (derived from the shikimic acid pathway), by an amide bond. Ochratoxin A (OTA) has been shown to be nephrotoxic, hepatotoxic, teratogenic and immunotoxic to several species of animals and to cause kidney and liver tumors in mice and rats (Więckowska, 2024).

OTA was first found in the Balkan region; however, it can be detected practically in all territories, it is accumulated in animal feed and in human food due to the favorable weather conditions and microclimate, and/or to improper storage of food components (Mousa, *et al*., 2021). It has also been proven to be carcinogenic in kidney and liver, and has been classified as a group 2B human carcinogen by the International Agency for Research on Cancer (IARC) and World Health Organization (WHO) (Barsouk,, 2021).

The wide occurrence of OTA and its high thermal stability makes the eradication of OTA from the food chain very difficult (Wang, 2023). In general, the average concentration of OTA is reported to range from 0.1 to 100 ng per gram of foodstuffs of plant origin. Several researches have revealed the ability of some microorganisms in the degradation of many toxic giant molecules. *Salmonella aureus* is not typically used in bioremediation. Instead, other bacteria like Pseudomonas, Bacillus, and certain strains of *E. coli* are more commonly employed for this purpose. Bioremediation involves using microorganisms to degrade or detoxify pollutants, such as pesticides and heavy metals, in the environment. These bacteria can metabolize harmful substances, converting them into less toxic forms and thereby cleaning up contaminated sites. (Petsas & Vagi 2019).

This study aims to conduct a preliminary assessment on the potency of gram-negative strain of *Salmonella aureus* species of bacteria in the biodegradation of Aflatoxins and Ochratoxins.

MATERIALS AND METTHOD

Sample Collection

The microbial sample was collected using a sterilized cotton swab from a rock on the hills that surrounds the old Dutse town (Garu) situated about 2km East-West direction (12.5922°N. 8.7334°E) from the Federal University Dutse, Jigawa State.

Collection of the bacterial sample

The sterilized swap stick was open and immersed into the sterilized water to moisturize it. It was then heat fixed on the glowing flame three times and then gently swab on the surface of the rock. It was then labeled as "Bacterial sample". The sample was kept in the refrigerator at about 4^0C until required for use.

Screening and Propagation of the Sample Colony

Preparation of enriched media

Thirty-two **(**32) grams of enriched agar was suspended in 1000 ml of distilled water sterilized at 121^0C for about 15 minutes in autoclave. The media was suspended in Petri dishes (20ml each). The sample was inoculated on the media by streaking in a lamina flow and incubated at 37^0C to obtain a sufficient growth. The bacterial colonies obtained were isolated into different petri dishes using the procedure above. Screening was done using Gram staining technique.

Culturing the fungal colony growth

To Nine (9) ml of distilled water in a test tube, was added 50mg of $NaNO_3$ followed by 1.0g of the powdered grain sample. The mixture was thoroughly shaken and allowed to settle. One (1.0) ml of the solution was evenly spread on two media dishes made from potatoes dextrose agar inside a lamina flow. The petri dishes were then covered and incubated at 27^0C. When sufficient growth of fungal colonies was attained, microscopic and morphological features were used to identify and isolate the *Aspergillus flavus and Ochraceus* respectively.

Culturing for Mycotoxin production

Yeast extract agar (YEA) was prepared by dissolving 72.5g of yeast agar powder into a conical flask containing 1010 ml of distilled water followed by addition of 18.75g of granulated table sugar and 0.5g of $MgSO_4$. The flask was vigorously shaken and then sterilized at 121^0C for about 15 minutes in an autoclave. Two petri dishes containing the prepared yeast agar was inoculated with the mycelia of *Aspergillus Ochraceus* obtained from the previous culture. The culture was incubated at 27^oC for three weeks in order to get adequate production of the respective mycotoxins.

Extraction of the fungal secondary metabolite (Mycotoxin)

Five (5) grammes of the fungal mycelium together with the agar media was weighed and placed in a test tube. Ten (10) ml of Dichloromethane was added and thoroughly votexed. The test tube was covered and allowed to stand for 1hr on a shaker. It was then filtered and the filtrate was dried using steam bath. The dried sample was reconstituted with 1.0ml of Dichloromethane and stored in a sample bottle until required for use.

Qualitative Determination of Aflatoxins and ochratoxins Using Thin Layer Chromatography (TLC)

In the cases of aflatoxins, 94ml of ether was mixed with 4.5ml of methanol and 1.5ml of distilled water in a test tube. The mixture was then introduced into the chromatographic tank and covered to allow the tank become saturated with the vapor of the mobile solution. Three spots marked A, B and C were made 1cm above the base of the TLC plate. Three drops of the sample extract were added on each mark and allowed to dry. The plate was then transferred into the TLC tank, covered and allowed stand until the solvent front nears the end of the plate. The plate was then removed, allowed to dry. Finally viewed under UV-light (in the UV-hood) to detect the presence of aflatoxins.

While for ochratoxin, 18ml of chloroform was mixed with 1.0ml of propan-2-ol and 1.0ml of ethyl acetate (90:5:5) in a test tube, the mixture was then introduced into the chromatographic tank and covered to allow the tank become saturated with vapour of the mobile phase. Procedure as described for aflatoxin above was also carried out.

Assessment of the Potency of Isolated *Salmonella aureus* Strain in the Degradation of the Extracted Aflatoxins and Ochratoxins.

Thirty-two **(**32) grams of enriched malt extract agar (MEA) were suspended in 1000 ml of distilled water sterilized at 121^0C for about 15 minutes in autoclave. The media was suspended in Petri dishes (20ml each). To the petri dishes, 1 mL of aflatoxin and ochratoxin extracts in the stock vials were each used to contaminate the prepared MEA agar. 1 ml of McFarland's standard of the bacterial sample was simultaneously inoculated on each of the contaminated agar plates. The respective contaminated agar plates were incubated for a period of 3 weeks at 28 ± 1^0C.

Extraction of mycotoxins after the bacterial action

The same extraction protocol for YEA previously described was also used/applied in this case, to assess the activity of bacterial strain on the fungal secondary metabolites (aflatoxins and ochratoxins) extracts.

Qualitative determination: Thin layer chromatography techniques previously described, was also applied in this case. The TLC plate was then visualized in the UV-hood to determine the presence of both aflatoxins and ochratoxins.

RESULTS

Dichloromethane (DCM) extract obtained from the whole lot of the YEA and the *Aspergillus flavus* mycelium (after 28 days) when subjected to TLC showed fluorescence characteristics of aflatoxin mycotoxin as indicated in PLATE I

PLATE1A: Image of the TLC chromatograph of aflatoxin produced by aflatoxigenic strain of *Aspergillus flavus* outside the UV hood.

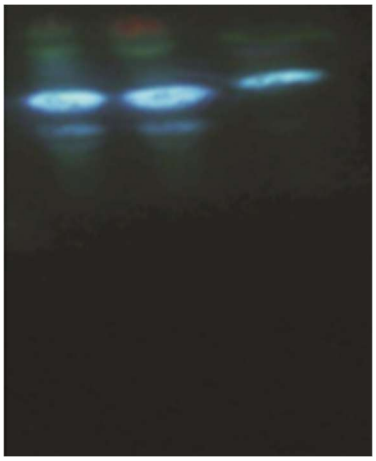

PLATE 1B: Image of the TLC chromatograph of aflatoxin produced by aflatoxigenic strain of *Aspergillus flavus* when subjected to UV Irradiation

However, when another freshly prepared agar media was contaminated with the initial filtrate containing the aflatoxin and thereafter inoculated with gram negative strain of the *Salmonella aureus* and incubated for three weeks, and then re-extracted again, a complete disappearance of the fluorescence was observed compared to what was initially observed. PLATE IIC & IID

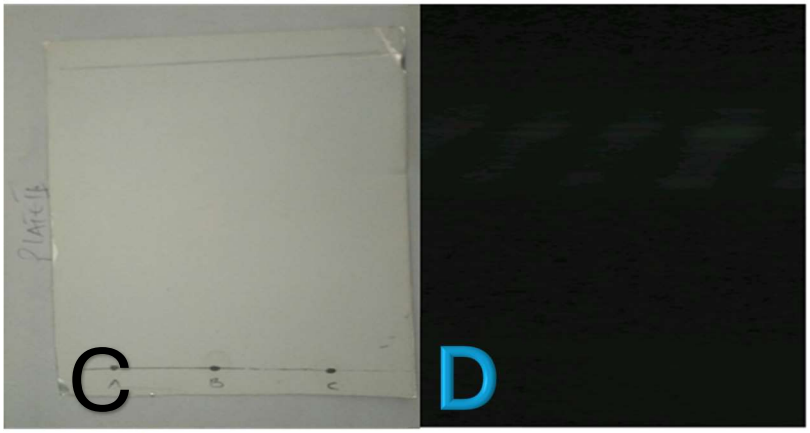

PLATE IIC: Image of the TLC chromatograph of a filtrate from an aflatoxin contaminated YEA incubated with a strain of *S.aureus* prior to exposure to UV radiation

PLATE IID: Image of the TLC chromatograph of a filtrate from an aflatoxin contaminated YEA incubated with a strain of *S.aureus* after exposure to UV radiation

More so, similar observation was made when strain of *Aspergillus parasiticus* was isolated and cultured on YEA and the eventually extracted with Dichloromethane (DCM). The sample extract was subjected to thin layer chromatography (TLC) and the plate (chromatograph) was viewed under Ultraviolet radiation in a UV-hood as presented in PLATE 1A & IB below.

PLATE IA: the image of TLC plate after first extraction prior to degradation by gram positive Salmonella aureus Prior to subjection to UV Irradiation

PLATE IB: the image of second chromatogram after subjection to UV irradiation

Similar trend on the lost (disappearance) of fluorescence was also observed after subjection of the extracted mycotoxin (ochratoxins) to the activity of the studied gram-positive *S. aureus*, PLATE IIC & IID

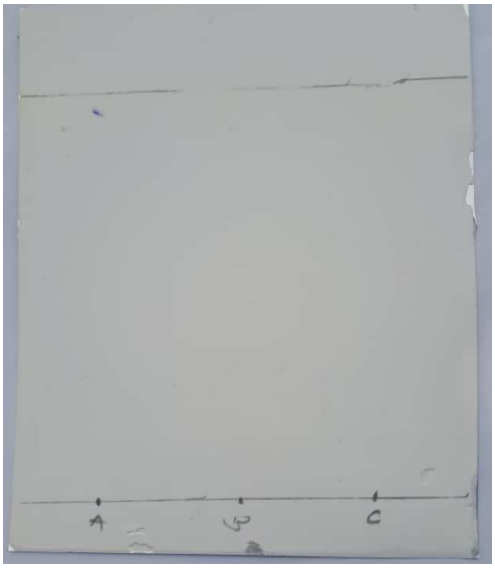

PLATE IIC: The image of TLC plate after subjecting the second extract to degradation by gram positive *Salmonella aureus*

PLATE IID: The image of second chromatogram after subjection to UV irradiation

DISCUSSIONS

As far as food safety and security is concerned, nothing can be more relieving and welcomed than identifying a new strain of a non-pathogenic organism with a unique characteristic of biodegradation or bioremediation of harmful toxins such as the mycotoxins. Bioremediation or degradation involves using microorganisms to degrade or detoxify pollutants, such as pesticides and heavy metals, in the environment. These microorganisms can metabolize harmful substances, converting them into less toxic forms and thereby cleaning up contaminated foods, feed or sites. (Petsas & Vagi 2019).

Although previous studies have not reported on the potency of *Salmonella aureus* in bioremediation or biodegradation of aflatoxins and ochratoxins, however, other bacteria like *Pseudomonas*, *Bacillus,* and certain strains of *E. coli* have been reported to be employed for this purpose. (Pandi *et al*., 2023). Moreso, considering the enormous number of microbial species in existence, sustained bio prospecting might lead to the discovery of some strains that can be more easily genetically manipulated compared to the identified thus far.

As indicated in PLATE1 (A and B) of this study, the fungal species being mycotoxigenic (aflatoxigenic) was observed to produce the mycotoxin. However, introduction of the isolated *S. aureus* strain (bacteria) into a sample agar

contaminated with this extracted mycotoxin resulted in the complete disappearance of this mycotoxin when the second extraction and qualitative analysis (TLC) was carried out as revealed in PLATE1 (C and D). Interestingly, similar scenario played out when the test organism (*S. aureus*) was introduced into an agar plate contaminated with ochratoxin.

Since there has been no report on the mycotoxin degradation activity of this bacterial species, it is most probable that changes induce by environment from which it was isolated or possibly climate change might have provoked changes in the genomic make up that lead to translation of a novel protein with such degradation potentials.

Moreover, the disappearance of the mycotoxins might be a second function of biosorption, biomineralisation, bioaccumulation and assimilation activities of some microbes which result in the masking of the mycotoxins, thereby rendering them undetected to the analytical method(s) employed.

Several researches have revealed the ability of some microorganisms to degrade many toxic giant molecules. Several bacterial strain, fungal strain, yeast and plants species were committed to this job. The process is broadly referred to as bioremediation and biodegradation depending on whether it occurs naturally or artificially. (King *et al.*, 2023)

A research group from General Electric laboratories characterized several strains of gram-negative bacteria that could degrade a wide range of poly chlorinated biphenyl congeners. Several groups of gram-positive *Rhodococcus* strains exhibit strong PCB degradation. (Vergani *et al.*, 2019).

More so, Silva *et al.* (2017), has reported the capability of *pseudomonas* spp to utilize hydro carbons as a carbon and energy sources and production of bisurfactant. They also pointed out in another study that, *Pseudomonas* spp has the capacity to reduce nitrate to nitrite and eventually to nitrogen gas by the denitrification pathway thereby providing the organism with a mode of respiration and ATP generation in the absence of oxygen. Kuroda, *et al.* (2021) also revealed the capability of *Streptomyces* spp to degrade DDT Pesticide of environmental concern. It has also been reported that various AZO dyes can be degraded aerobically by *spongomanas spp.* (Kumar *et al.*, 2021)

From the foregoing studies, it is pertinent to point out that microorganisms have been playing pivotal role in the biodegradation or bioremediation of toxic substances. However, the set back of all these successes achieved is, origin and availability of these mentioned microbes in our own local environment. It therefore becomes imperative to search for a strain that is non-pathogenic and readily available in our local environment for easy accessibility and deployment to solve our immediate problem within our immediate environment.

From the result obtained in this study, it can be concluded that gram-negative strain of the *S. aureus* obtained from the immediate rocky surroundings of Dutse, having clear non-pathogenic features can be a possible candidate to be deployed in the mitigation of aflatoxin and ochratoxins contamination. Various reports have indicated that these toxins have proven to be carcinogenic, teratogenic, hepato-toxic and nephrotoxic. (Nazareth, *et al.*, 2024)

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EVALUATION OF THE ORGANOLEPTIC ATTRIBUTES OF TURMERIC (*Curcuma longa)* FORTIFIED GROUNDNUT CAKE

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ABSTRACT
Turmeric (*Curcuma longa*) is known for its potent bioactive compounds, particularly curcumin, which have numerous health benefits. This study evaluates the organoleptic attributes of groundnut cake (GNC) fortified with turmeric, exploring its sensory qualities and potential as a functional food ingredient. Control (0 g turmeric /100 g GNC (S1), 2 g turmeric/100 g GNC (S2), 4 g turmeric/100 g GNC (S3), 6 g turmeric/100 g GNC (S4), and 8 g turmeric /100 g GNC (S5). Sensory analysis was carried out to assess the impact of turmeric powder on colour, appearance, flavour, aroma, texture, and overall acceptability of groundnut cake, utilizing a 9-point hedonic scale with evaluations conducted by thirty-three semi-trained panelists. The result of the colour was significantly higher in S3 (7.74) than S2 (7.49), when compared to the control S1 (6.77), while S5 (7.37) and S4 (7.34) were statistically similar. In the terms of appearance, S2 (7.37) was significantly better than S4 (7.03) with the least value. With regards to flavour, S2 (7.06) was significantly higher when compared to S5 (5.91) with the least value, while S1(6.55) and S3 (6.46) were statistically similar and higher than S4 (5.94). The S2 (6.79) for aroma has significant improvement when compared to S5 (5.92) with the least value. Texture, S2 (6.85) was significantly better when compared to S4 (6.19) with the least value. The overall acceptability for control group S1 7.29 which was significantly higher when compared to S5 (6.33) with the least value. The result showed that the inclusion of 2 g turmeric powder per 100 g of GNC enhanced all the organoleptic attributes. It is therefore recommended that 2 g/100 g of GNC should be used for improving consumer preference and overall acceptability of this food product.

Keywords: Turmeric, groundnut cake, fortified, organoleptic attributes,

INTRODUCTION
Turmeric (*Curcuma longa*), is a spice that has distinctive flavour, vibrant colour, and numerous health benefits, including antioxidant, anti-inflammatory, and antimicrobial properties (Chakraborty *et al.,* 2023). Its active compound, curcumin, has gained significant attention for its potential applications in functional foods due to its therapeutic effects in mitigating chronic diseases such as arthritis, cardiovascular diseases, and cancer (Patel *et al.,* 2022). Groundnut cake, a by-product of groundnut oil extraction, is a rich source of protein, fibre, and essential nutrients, traditionally used as animal feed but increasingly explored for human consumption (Adeyemi *et al.,* 2021).

The fortification of groundnut cake with turmeric offers an innovative approach for enhancing its nutritional profile while enhancing its organoleptic qualities. Sensory attributes such as taste, colour, texture, and aroma are crucial factors influencing consumer preference, acceptability and marketability (Musa *et al.,* 2020). Therefore, evaluating the impact of turmeric on these sensory characteristics is vital for determining its potential to improve the acceptability of groundnut cake as a functional food (Rasool *et al.,* 2021). This study aims to assess the organoleptic properties of turmeric-fortified groundnut cake and explore its acceptability among consumers, thereby contributing to the development of nutrient-dense food options for diverse populations.

MATERIALS AND METHODS
Sources of raw material
Groundnut seed (*Arachis hypogea*) and turmeric rhizome (*Curcuma longa)* used for this study were purchased from Kure-ultra modern market in Bosso Local Government Area Minna, Niger State.

Preparation of turmeric rhizome powder
The turmeric rhizomes were sorted, thoroughly washed with water to remove soil particles on them. They were further peeled, thinly grated and sundried for six hours each day for two days making a total of twelve hours sun drying. After drying, they were ground into powder and stored in a plastic air tight container.

Production of groundnut cake

Groundnut cake was prepared according to the method described by Chibuzo and Ali (1994). Groundnuts were sorted, washed, sundried and roasted slightly for about 15 minutes under medium heat. The roasted groundnut was cooled and the coats were removed by manual abrasion. Afterwards the roasted groundnut was grinded into paste with the use of harmer mill. Little quantity of warm water was then added gradually to the groundnut paste and stirred until oil oozed out, which took about 30 minutes. The groundnut paste samples were then fortified with varying concentrations of turmeric per 100g of groundnut cake (GNC) control (S1) 0 g turmeric/100 g of GNC, 2 g (S2) turmeric/100 g GNC, 4 g (S3) turmeric/100 g GNC, 6 g (S4) turmeric/100 g GNC, and 8 g (S5) turmeric/100 g GNC. The oil was drained out and put in a separate container. Turmeric was then added to the groundnut paste flattened on a rolling board, cut in small sizes and fried in the extracted oil until a golden-brown colour was obtained. The cake was then drained and cooled. Each sample were fried and stored separately in a labeled air-tight container

Assessment of the organoleptic attributes of groundnut cake fortified with turmeric rhizome powder

The sensory evaluation was conducted using 33 semi-trained panelists from the Federal University of Technology, Minna, campus community. The evaluation followed the method described by Grunert *et al.* (2004), utilizing a 9-point hedonic scale with the following ratings: 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, and 1 = dislike extremely.

The panelists assessed the groundnut cake samples based on sensory attributes, namely colour, appearance, texture, taste, aroma and overall acceptability. After tasting each sample, panelists were provided with cracker biscuits and water to cleanse their palates and prevent flavour carryover between samples.

RESULTS

Effect of the Inclusion of Turmeric in Groundnut Cake on Organoleptic Attributes

The results on the effect of the inclusion of turmeric on the organoleptic attributes of groundnut cake is shown in Table 1. The result showed that there were significant differences ($p<0.05$) in all the parameters evaluated. It was evaluated using nine-point hedonic scale with the help of thirty-three semi-trained taste panelists. The result of the colour was significantly higher in S3 (7.74) than S2 (7.49), when compared to the control S1 (6.77), while S5 (7.37) and S4 (7.34) were statistically similar. In the terms of appearance, S2 (7.37) was significantly better than S4 (7.03) with the least value. With regards to flavour, S2 (7.06) was significantly higher when compared to S5 (5.91) with the least value, while S1(6.55) and S3 (6.46) were statistically similar and higher than S4 (5.94). The S2 (6.79) for aroma has significant improvement when compared to S5 (5.92) with the least value. Texture, S2 (6.85) was significantly better when compared to S4 (6.19) with the least value. The overall acceptability for control group S1 7.29 which was significantly higher when compared to S5 (6.33) with the least value.

Table 1: Organoleptic Attributes of Turmeric Fortified Groundnut Cake

Parameters	Samples							
	S1	S2	S3	S4	S5	SEM	P-value	Sig
Colour	6.77^{c}	7.49^{b}	7.74^{a}	7.34^{b}	7.37^{b}	0.10	0.00	*
Appearance	6.74^{c}	7.37^{a}	7.20^{ab}	7.03^{b}	7.09^{b}	0.23	0.00	*
Flavour	6.55^{b}	7.06^{a}	6.46^{b}	5.94^{c}	5.91^{c}	0.46	0.00	*
Aroma	6.42^{b}	6.79^{a}	6.31^{bc}	6.06^{cd}	5.92^{d}	0.33	0.00	*
Texture	6.44^{bc}	6.85^{a}	6.54^{b}	6.19^{c}	6.30^{bc}	0.27	0.01	*
Overall acceptability	7.09^{b}	7.29^{a}	6.79^{b}	6.45^{d}	6.33^{d}	0.38	0.00	*

This was determined by using hedonic scale 1-9: 1=dislike extremely, 2=dislike extremely, 3=dislike moderately, 4 dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely. Values with different superscript across the row are significantly ($p < 0.05$) different. SEM = Standard error of mean; P-value = Probability value; Sig = Significance; * = Significant; NS = Not significant.

DISCUSSION

The inclusion of 2 g of turmeric per 100 g of groundnut cake (S2) showed significant improvements in various organoleptic attributes compared to other inclusion levels. The results showed that, it enhanced mouthfeel, appearance, flavour, aroma, and texture which led to higher overall acceptability for (S2) that contains 2 g of turmeric in groundnut cake, indicating broad consumer preference (Lopez *et al.*, 2021). The inclusion of turmeric significantly improved the colour of groundnut cake, which is consistent with previous findings that turmeric enhances the colour of food products (Sanchez *et al.,* 2013). The natural pigments in turmeric likely enhanced the groundnut cake colour, improving its visual

appeal (Davis *et al*., 2020). This suggests that excessive turmeric may negatively impact mouthfeel and flavour quality. This might be due to the bitter and astringent properties of turmeric at higher concentrations (Hossain and Ishimine, 2021). A similar study found that high levels of spice blends can decrease mouthfeel and flavour scores (Jensen *et al.,* 2015). A moderate amount of turmeric improves the appearance, and aroma of groundnut cake, while the overall acceptability makes its more attractive to consumers (Kumar *et al.,* 2018). The optimal inclusion of turmeric (2 g) did better than the control while balancing the flavour, aroma, and colour enhancements which minimize negative effects. Turmeric contains curcumin, which has antioxidant and anti-inflammatory properties that can enhance food quality (Menon, 2023). The inclusion of 2 g of turmeric per 100 g of groundnut cake (S2) is the optimal level for enhancing organoleptic attributes and consumer acceptance. This study supports the use of turmeric as a natural food additive to improve the sensory quality of groundnut cake.

CONCLUSION AND RECOMMENDATIONS

In this study it was established that the inclusion of 2 g of turmeric/ 100 g of GNC (*S2*) significantly enhanced the sensory attributes of groundnut cake, especially colour, flavour, and texture, turmeric concentration above 2 g reduced the perception of sensory attributes. It is therefore recommended to use turmeric at 2 g/100 g of GNC for optimal sensory appeal, consumer preference and overall acceptability.

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PHYTOCHEMICAL, NUTRITIONAL AND ANTIOXIDANT PROPERTIES OF PUMPKIN LEAF AND FRUIT (*Cucurbita Maxima*) FROM BOSSO-NIGER STATE, NIGERIA

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ABSTRACT
Cucurbita maxima commonly known as pumpkin, is widely cultivated throughout the world for use as vegetable as well as medicine plant. Phytochemical analysis, nutritional and antioxidant properties of *Cucurbita maxima* leaf and fruit methanol extracts were determined in this study. The phytochemical and proximate analysis were performed according to standard method. The antioxidant activity was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP). The qualitative phytochemical screening of *Cucurbita maxima* fruit and leaf revealed that Alkaloids (9.07±0.01), Tannins (4.32±0.01), and terpenoids (8.64±0.01) were found to have the highest concentration in the fruit while saponins (8.64±0.01), flavonoids (3.86±0.01) and steroids (3.86±0.01) were found to be higher in the leaf. The proximate composition of pumpkin leaf and fruit showed significant difference in moisture (10.65 ± 0.04) (8.07 ± 0.01), fibre (2.03 ± 0.03) (4.16 ± 0.03), ash contents (3.08 ± 0.08) (2.17 ± 0.05), fat (5.12 ± 0.20) (2.07 ± 0.20), (67.99± 0.03) (73.12± 0.01), and protein (10.580.06) (9.72± 0.07) of the pumpkin leaf and fruit respectively. Both extracts exhibited a dose dependent antioxidant activity in DPPH and FRAP assays. The *Cucurbita maxima* extracts exhibited significantly ($p < 0.05$) lower antioxidant activities compared to the standard (ascorbic acid). The results of mineral analysis of pumpkin shows that potassium (48.79±0.01 mg/g) and iron (33.60±0.00 mg/g) contents were higher in the pumpkin methanol fruit extract, followed by sodium (17.88±0.04 mg/g) while potassium (41.57±0.04 mg/g) and zinc (22.30±0.15 mg/g) was the most prominent element followed by iron (8.14±0.00 mg/g) in the pumpkin methanol leaf extract. These results suggest that *Cucurbita maxima* could serve as a valuable source of nutrients and antioxidants, particularly in resource-constrained communities.

Keywords: *Cucurbita maxima*, Phytochemicals, Proximate composition, Antioxidant properties and Mineral analysis.

INTRODUCTION
Oxidative stress has been implicated in the pathogenesis and progression of several diseases such as atherosclerosis, diabetes mellitus, hypertension, cardiovascular diseases, neurodegeneration, autoimmune diseases lung, pancreatic, kidney disorders and cancer to mention a few (Talebi *et al*., 2021). Stress related disorders have become epidemic in developing and under-developed countries. Conventional therapeutic strategies mostly attempt to relieve the clinical manifestations of these disorders and their complications. However, studies have shown that they tend to increase toxicity leading to damage of sensitive organs (Rahman *et al.,* 2012; Jahan *et al.,* 2023).

In light of this, the use of complementary medicines for conditions linked to oxidative stress has grown, and plant-based antioxidant therapies are now common in the majority of developing nations (Mohan *et al.,* 2013). Several plant extracts and their secondary metabolites are being investigated for their antioxidant activities since antioxidants are essential in reducing oxidative stress-related diseases (Gomathi *et al.,* 2017, Moscolo *et al*., 2024). Using plant-based antioxidants is crucial for preventing the body's oxidation-induced signaling pathways from being activated (Sies *et al.,* 2020; Talebi *et al*., 2021).

The consumption of nutritive local foodstuffs will help to supplement the nutrients of the staple carbohydrate foods of the poor who cannot afford enough protein foods of animal origin (Hussain *et al.,* 2022a) and people living with degenerative diseases. The use of available local food sources is increasingly pursued, and many reports on some lesser-known seed and fruit such as pumpkin *(Cucurbita maxima),* indicated that they could be good source of nutrients and medicinal compounds for both man and livestock (Jahan *et al.,* 2023, Duvbiama *et al*., 2023).

Cucurbita maxima (Pumpkin) is an angiosperm plant belonging to the family *Cucurbitaceae* and genus *Cucurbita* and their usage as a traditional food treating diseases has been widely reported (Omoraye and Dilworth, 2020; Hussain *et al.,*

2022b; Duvbiama *et al.*, 2023). In traditional medicine, it is known to exhibit many health benefits which include prevention of growth and reduction of size of prostrate, reduction of bladder and urethral pressure and alleviates diabetes (Batool *et al.*, 2022; Hussain *et al.,* 2022b). Pumpkin is a versatile vegetable having identical position among all vegetables, due to its peel, flesh and seeds, each possessing outstanding phytochemicals applicable in treatment and prevention of medical disorders (Sharma *et al.*, 2020; Hussain *et al.,* 2021). *Cucurbita maxima* has also exhibited anthelmintic, antihypertensive, anticancer, antibacterial, and anti-inflammatory properties (Saha *et al.,* 2019). Pumpkin is a very common vegetable widely consumed by many people not only in Nigeria but all over the world (Kim *et al.,* 2012, Lestari and Meiyanto, 2018). While several studies have focused on the nutritional value of *Cucurbita maxima* flesh and seeds, there is limited information available on the phytochemical and nutritional profiles of the leaves. Moreso, this plant fruit and leaf is mostly consumed by many people in Nigeria without knowledge of its nutritional compositions. Therefore, it is important to ascertain the nutritional profile of locally obtained *Cucurbita maxima* in Nigeria. In this context the present study was conducted to explore the preliminary phytochemical, proximate compositions and antioxidant properties of pumpkin leaves and fruit.

MATERIALS AND METHODS

Sample collection and preparation

The pumpkin leave and fruits were collected from the natural habitat of Bosso village, Bosso Local Government, Niger State, Nigeria. The pumpkin samples were authenticated at the Biological Sciences department of Federal University of Technology, Minna where it was allocated a voucher no: (FUT/PLB/CONVO/001). The leaves and the fruits were sliced and crushed. The samples extraction was performed using cold maceration for 72 hours with absolute methanol. The samples were then filtered using filter paper and the filtrate was concentrated in a rotary evaporator and kept in airtight polyethylene bags in a refrigerator for further analysis.

Reagents

Some of the reagents used in this research includes, Distilled water, Folin-Ciocalteu's reagent, methanol, 2,2,-Diphenyl-1-picrylhydrazyl (DPPH) and sulphuric acid. All the chemicals and solvents used in this experiment were of good analytical grade

Phytochemical Screening of the sample(s)

Determination of Total Phenol Singleton *et al.*, (1999)**,** Total flavonoid content (Chang *et al.,* 2002), Total alkaloid and saponins, Oloyed, (2005), Tannin content (AOAC, 2005).

Proximate Analysis of the sample(s)

The proximate analysis was carried out for moisture content, ash content, crude fibre, fat content, protein and carbohydrates were carried out using AOAC, 2019 methods:

Determination of Moisture Content

Five grams (5g) of the sample was weighed into a previously weighed moisture can. The sample in the can was dried in the moisture extractor at 105^{o}C for 3 hours. It was cooled in a dessicator and weighed. It was then returned to the oven for further drying. Drying, cooling, and weighing were done repeatedly at an hour interval until there were no further diminutions in the weight (i.e. a constant weight was obtained). The weight of moisture loss was calculated and expressed as a percentage of the weight of sample analysed.

$$\%\ moisture\ content = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad \text{.....Equation 1}$$

Where; W_1 = Weight of empty can

W_2 = Weight of empty can + sample before drying

W_3 = Weight of can + sample dried to a constant weight

% total solid (Dry matter) = 100 - % moisture content

Determination of Ash Content

Five grams (5g) of the processed sample was measured into a previously weighed porcelain crucible. The sample was burnt to ashes in a muffle furnace at 550^{O}C. When completely ashed, it was cooled in a desiccator and weighed. The weight of ash obtained was calculated by difference and expressed as a percentage of the weight of sample analysed.

$$\%\ \text{Ash content} = \frac{W2 - W1}{Weight\ of\ sample} \times 100 \quad \text{.....Equation 2}$$

Where; W_1 = weight of empty crucible
W_2 = weight of crucible + Ash

Determination of Crude Fibre

Five grams (5g) of the processed sample was boiled in 150 ml of 1.25% H_2SO_4 solution for 30minutes under reflux. The boiled sample was washed in several portions of hot water using a two-fold muslin cloth to trap the particles. The residue was returned to a flask and boiled again in 150ml of 1.25% NaOH for another 30min under the same condition. After washing in several portion of hot water, the sample was allowed to drain before it is transferred to a weighed crucible where it was dried in the oven at 105°C to a constant weight. It was thereafter taken to a muffle furnace in which it was burnt until only ash was left of it. By difference, the weight of fibre was obtained and expressed as a percentage of the weight of sample analysed.

% crude fibre = $\frac{W2-W3}{Weigh\ \ of\ sample} \times 100$ ……Equation 3

Where, W_2= weight of crucible + sample after boiling, washing and drying.

W_3 = weight of crucible + sample ashing.

Determination of Fat Content

One gram (1g) of sample was wrapped in a previously weighed porous paper (Whatman No 1 filter paper) and placed in a clean dry soxhlet reflux flask. The flask was mounted unto an extraction flask containing 300 ml of hexane. The upper end of the reflux flask was connected to a water condenser. On heating the extraction flask with a non-luminous heat source (hot plate), the solvent boiled, vaporized, and condensed into the reflux flask and covered the wrapped samples. The sample remained in contact with the solvent until the reflux flask filled up and siphoned over thereby carrying extracted oil (fat) down to the boiling flask. The cycle of vaporization, condensation, extraction, and reflux siphon was allowed to go on repeatedly for fourteen times (4h). The defatted wrapped samples were removed (with the aid of pair of forceps) and dried in the oven at 100°C for 30min after which they were cooled in a desiccator and weighed. By difference, the weight of oil (fat) lost was calculated and expressed as a percentage of the sample weight.

% fat = $\frac{W2-W3}{W2-W1}$ x 100 ……Equation 4

Where; W_1 = weight of empty filter paper
W_2 = Weight of paper + sample before defatting
W_3 = weight of paper + sample after defatting

Crude Protein Determination

The total nitrogen was determined and multiplied with factor 6.25 to obtain the protein content. Half gram (0.5g) of the sample was mixed with 10 ml of concentrated H_2SO_4 in a digestion flask. A tablet of selenium catalyst was added to it before it was heated under a fume cupboard until a clear solution was obtained (i.e. the digest). The digest was diluted to 100 ml in a volumetric flask and used for the analysis. 10ml of the digest was mixed with equal volume of 45% NaOH solution in a Kjeldahl distillation into 10ml of 4% boric acid containing three drops of mixed indicator (bromocresol green/methyl red). A total of 50 ml of distillates was collected and titrated against 0.02N EDTA from green to a deep red end point. A reagent blank was also digested, distilled, and titrated. The nitrogen and protein contents were calculated using the formula below:

% protein = % N_2 × 6……...Equation 5

$$\%N_2 = \frac{100}{10} \times \frac{N \times 14}{1000} \times \frac{Vt \times T - B}{Va}$$

Where; W = Weight of sample (0.5g)

Vt = Total digest volume (100ml)

Va = Volume of digest analysed (10ml)

T = Sample titre value

B = Blank titre value

Carbohydrate Determination

This was determined by the difference method (James, 1995). The calculation is given by the equation:

$$\% \text{ Carbohydrate} = 100 - (M+P+F_1+A+F_2) \quad \text{.....Equation 6}$$

Where P = Protein

F_1 = Fat

A = Ash

F_2 = Fibre

Antioxidant Assays

Determination of free radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging assay as described by Oyaizu (1986) as described by Madaki *et al.,* 2019. Briefly, different concentrations of extracts and ascorbic acid (62.5, 125, 250 and 500 µg/mL) were prepared from stock solutions (1000 µg/mL), prepared by weighing and dissolving 0.01g of the extracts and ascorbic acid, respectively in 10 mL of methanol. Thereafter, 2mL of 0.004% DPPH in methanol added to 1 mL of various concentrations of plant extracts and ascorbic acid, respectively. The reaction mixtures were incubated at 25°C for 30 minutes. The absorbance of each test mixture was read against blank at 517 nm using double beam Shimadzu UV-1800 series spectrophotometer. The experiment was performed in triplicates. The percentage antioxidant activity was calculated using the formula below:

$$\% \text{ Inhibition} = \frac{\text{Ablank} - \text{Asampl}}{\text{Ablank}} \times 100 \quad \text{.....Equation 7}$$

Determination of antioxidant activity by FRAP (Ferric Reducing Antioxidant Power) Assay

Estimation of antioxidant activity of the plant extracts via ferric reducing antioxidant power assay was conducted according to the method of Oyaizu (1986) as described by Madaki *et al.,* 2019. Stock solutions of plant extracts and ascorbic acid (1000 µg/mL) were prepared, from which different concentrations 62.5, 125, 250 and 500 µg/mL were prepared. In this assay, 1 mL of each plant extracts and ascorbic acid concentration was mixed with 1 mL of 0.2 M sodium phosphate buffer and 1 mL of 1% potassium hexacyano ferrate (III). The reaction mixtures were incubated at 50 °C for 20 minutes. Thereafter, 1 mL of 10% TCA was added. The reaction mixtures were then centrifuged for 10 minutes at room temperature. Then 1 mL of each supernatant obtained was mixed with 1 mL of distilled water and then 0.2 mL of 0.1% ferric chloride was added. The blank was prepared in the same extracts as samples except that the extracts were replaced by distilled water. The absorbance of the test mixtures was read at 700 nm. The percentage antioxidant activity was calculated using the formula below:

$$\% \text{ activity} = \frac{Asample - Ablank}{Asample} \times 100 \quad \text{----------Equation 8}$$

Mineral Analysis

The samples of pumpkin leaf and fruit were digested into solution by wet digestion using a mixture of concentrated Nitric, perchloric and sulphuric acids in the ratio 9:2:1 respectively. Fe, Zn, Mg, and Ca were determined by Atomic Absorption Spectrophotometer (Model Accusy 211 Bulk Scientific USA), sodium and potassium by flame photometer (Model FP6410 Harris Medical Essex, England), (AOAC, 2019) as described Madaki *et al.* (2016).

Statistical Analysis

Data collected were subjected to one-way Analysis of Variance (ANOVA) using Completely Randomized Design (CRD) of SPSS package and means separated by Duncan's Multiple Range Test (Duncan,2010) using the same computer package.

RESULTS

Quantitative phytochemical Analysis of Pumpkin Leaf and Fruit

Table 1 indicate the quantitative phytochemicals estimated in the methanol leaf and fruit extracts of pumpkin. Terpenoids, Alkaloids and Tannins were found to have the higher concentrations in the fruit compared to the leaf while, Saponins, Flavonoids, Phenols and Steroids were found to be higher in the leaf.

Table 1: Quantitative Phytochemical Constituents of the Methanol Extract of Pumpkin Leaf and Fruit

Phytochemical constituents	Concentration (mg)	
	Leaf	Fruit
Terpenoids	4.06±0.01	8.64±0.01
Alkaloids	8.62±0.01	9.07±0.01
Saponins	4.13±0.01	3.87±0.01
Tannins	3.96±0.01	4.32±0.01
Steroids	4.13±0.01	3.10±0.01
Glycosides	-	-
Phenols	3.26±0.01	1.02±0.01
Flavonoids	3.86±0.01	1.05±0.01

Results are expressed as mean ± SEM (n = 3).

Proximate Compositions of the Leaf and Fruit of Pumpkin

The mean values of the proximate compositions for the pumpkin leaf and fruit are presented in the tables 2. The results revealed that the moisture, protein, fat, fiber, ash, protein, and the carbohydrate contents of the pumpkin (leaf and fruit) were significantly different ($p<0.05$) in both plant parts.

Table 2: Proximate Compositions of the Pumpkin Leaf and Fruit

Proximate compositions	Leaf (%)	Fruit (%)
Moisture content	8.07 ± 0.01^b	10.65 ± 0.04^a
Ash content	3.08 ± 0.08^b	2.17 ± 0.05^b
Fat content	5.12 ± 0.20^b	2.07 ± 0.20^a
Fiber content	2.03 ± 0.03^b	4.16 ± 0.03^a
Protein content	10.58 ± 0.06^a	9.72 ± 0.07^b
Carbohydrate content	67.99 ± 0.03^b	73.12 ± 0.01^a

Values are mean ± standard error of mean (SEM) of triplicate values (n=3). Mean values across the row, with different letters as superscripts are considered significant at ($p < 0.05$)

2,2, -Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity of Pumpkin Leaf and Fruit Methanol Extracts

The table 3 below shows that the percentage (%) inhibition of DPPH radical by the plant extracts at different concentration increased with increase in concentration of the extract for the leaf and fruit. The plant extracts exhibited lower antioxidant activity compared to standard (ascorbic acid).

Table 3: 2,2, -Diphenyl-1-picrylhydrazyl scavenging activity of Methanol Extracts of Pumpkin Leaf and Fruits

Concentration (μg/mL)	Methanol extract		Standard (Ascorbic acid)
	Leaf	Fruit	
500	66.23 ± 0.94^b	67.63 ± 0.82^b	96.38 ± 0.09^a
250	48.07 ± 0.04^a	50.70 ± 0.01^a	93.52 ± 0.02^b
125	29.90 ± 0.04^c	33.71 ± 0.32^b	86.95 ± 0.01^a
62.5	17.09 ± 0.07^c	19.03 ± 0.07^a	74.02 ± 0.01^c

Values are expressed as mean ± SEM. Mean values across the row, with different letters as superscripts are considered significant at ($p < 0.05$).

Ferric Reducing Antioxidant Power of Pumpkin Leaf and Fruit Methanol Extracts

The table 4 shows the ferric reducing power of methanol extract of the pumpkin leaf and fruit. The percentage (%) inhibition of FRAP radical by the plant extracts was in dose dependent pattern. The plant extracts exhibited lower antioxidant activity compared to standard (ascorbic acid) with highest activity achieved at 500 μg/mL.

Table 4: Ferric reducing antioxidant power activity of Methanol Extracts of Pumpkin Leaf and Fruits

Concentration (µg/mL)	Methanol extract		Standard (Ascorbic acid)
	Leaf	Fruit	
500	68.21± 0.84^{ab}	69.92±0.72^{a}	97.88± 0.09^{a}
250	50.51± 0.04^{c}	54.75±0.11^{a}	96.43± 0.02^{c}
125	24.09± 0. 44^{a}	31.15±0.35^{b}	92.67± 0.02^{ab}
62.5	11.86± 0.57^{c}	20.03±0.67^{a}	78.81± 0.51^{c}

Values are expressed as mean ± SEM $p<0.05$.

Selected Mineral Composition of Methanol Extracts of Pumpkin Leaf and Fruits

The table 5 shows the concentration of selected mineral content of the pumpkin leaf and fruit methanol extracts. Higher concentrations of sodium, magnesium, potassium, calcium and iron were estimated in methanol fruit extract while zinc was higher in methanol leaf extract.

Table 5: Selected Mineral Composition of Methanol Extracts of Pumpkin Leaf and Fruits

Mineral	Amount (mg/g)		P value
	PF	PL	
Na	17.88±0.04^{b}	2.81±0.01^{a}	0.00
Mg	8.27±0.01^{b}	0.19±0.00^{a}	0.00
K	48.79±0.01^{b}	41.57±0.04^{a}	0.00
Ca	3.77±0.03^{b}	2.02±0.01^{a}	0.00
Fe	33.60±0.00^{b}	8.14±0.00^{a}	0.00
Zn	2.45±0.03^{a}	22.30±0.15^{b}	0.00

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values across row with different superscripts are significantly different at $p < 0.05$. Where, PF=Pumpkin fruit; PL= Pumpkin leaf

DISCUSSION

The use of traditional herbal remedies as preventive measures and to cure certain diseases are very common in developing and develop countries due to their lesser side effects with fewer complications (Hussein *et al*., 2022a). Extracts of different herbs, fruits and vegetables have been found loaded with biologically active components and are a well alternative source of drugs (Mohammed *et al*., 2020). This study revealed the nutritional composition including proximate analysis (ash, moisture, protein, fat, fiber, and carbohydrate) and mineral contents, of fruit and leaf, parts of the pumpkin (*Cucurbita maxima*) plant.

The result of this study shows that the qualitative and quantitative phytochemical screening of methanol extracts of pumpkin leaf and fruit contain various phytochemicals including flavonoids, alkaloids, phenol, tannins, terpenoids, and saponins while glycosides were not observed. Muhammed *et al*. (2020) and Halder *et al*. (2022) reported similar phytochemical constituents in their studies of pumpkin leaf and peel respectively. It was also observed that alkaloids have the highest concentration in both the leaf and fruit methanol extracts while the lowest concentration was recorded for phenol in both plant extracts. These phytochemicals possess strong antioxidant activities and exhibit antimicrobial, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and antiviral activities (Sharma *et al*., 2018). For instance, Alkaloids are reported to possess therapeutic potential in various mood disorders and neurodegenerative diseases (Hussain *et al*., 2018).

Analysis of nutritional composition is important as it is necessary to understand the quality and the health-beneficiary effects of food or food products (Abou-Elella and Mourad, 2020). The proximate analysis of *C. maxima* showed significantly ($p < 0.05$) higher percentages of carbohydrates and fiber in the fruit when compared to the leaf extract while percentage moisture, protein, fat and ash contents were higher in *C. maxima* Methanol leaf extract which is related to the work of Omimakinde *et al*., 2019. The carbohydrate composition (67.99± 0.03 %; 73.12± 0.01%) of *C. maxima* leaf and fruit methanol extracts recorded were high, whereas proteins was the second most abundant component in the leaf and fruit has crude fat as the second most prominent which is an indication that the leaves and fruits are a good source of energy to both humans and animals. The values obtained were higher than those reported by Omimakinde *et al*. (2019) (about 51 and 64% in Leaf and Pod, respectively). Crude fat contents of leaf and fruit were very low (2.03 ± 0.03 % and 4.16 ± 0.03 %, respectively) suggesting that regular consumption of pumpkin is healthy cannot lead to

obesity. Moisture content is used as a measure of susceptibility to microbial action or contamination (Uyoh *et al.,* 2013). The relatively low concentration of moisture indicates that their dried leaf may not easily be susceptible to microbial spoilage when preserved. The low moisture content will drastically slow down the development of microorganisms and hinder the hydrolysis of component material (Ngaha *et al.,* 2020). The appreciable amounts of proteins in leaf and fruit shows that they can be used in the human diet to supplement or meet protein needs and reduce poverty and malnutrition among the poor who cannot afford protein rich foods such as meat and fish (Okonya and Maass, 2014). The high amount of protein, especially in leaf justifies its uses in cooking, as the edible parts of pumpkin might help an individual to meet the daily recommended intake of macronutrients (Jahan *et al*., 2023)

The antioxidant potential of a plant or its part, depends upon the presence of biological active ingredients capable of suppressing free radicals and reactive oxygen species in living body complications (Hussein *et al*., 2022). DPPH free radical scavenging activity of pumpkin leaf and fruit methanol extracts was found to increase at dose dependent manner with highest activity (66.23± 0.94; 67.63±0.82) observed at 500 µg/mL concentration**,** which is in contrast with the study conducted by Hussain *et al.* (2021), in which a comparison of antioxidant and antimicrobial activities of pumpkin peel, flesh and seeds was made. In addition, the pumpkin fruit methanol extract exhibited significantly ($p<0.05$) higher antioxidant activity. Similar result was also obtained in the ferric oxide reducing power activity. The results of this research are consistent to those reported by Kabbashi *et al.* (2014). In their study, they reported high antioxidant activity of ethanolic seed extract of *C. maxima*. This indicates that pumpkin not only being used as food in various communities across the globe, but it can also act as an important raw material for drug development in pharmaceutical industries.

Minerals fulfil a wide variety of functions in the optimal functioning of the immune system. The supply of minerals is important for the optimal function of the innate immune system as well as for components of adaptive immune defense; this involves defense mechanisms against pathogens in addition to the long-term balance of pro- and anti-inflammatory regulation (Weyh *et al.,* 2022). The results of mineral analysis of pumpkin shows that potassium (48.79±0.01 mg/g) and iron (33.60±0.00 mg/g) contents were higher in the pumpkin methanol fruit extract, followed by sodium (17.88±0.04 mg/g) while zinc (22.30±0.15 mg/g) was the second most prominent element followed by iron (8.14±0.00 mg/g) in the pumpkin methanol leaf extract. However, zinc recorded the lowest mineral composition in pumpkin methanol fruit extract while magnesium (0.19±0.00 mg/g) was the lowest in pumpkin methanol leaf extract estimated. It was observed that concentrations of most elements were higher in the fruit compared to the leaf with exception of zinc. These results contradict the work of Elinge *et al.* (2012) where higher elemental concentrations were reported in their study of mineral compositions of pumpkin seed extract, this could be due to variation in plant parts, vegetation and solvent of extraction. Zinc is a very important mineral responsible for enhancing metabolism function and immune system. Pumpkins are excellent source of an important mineral Zn, which plays a vital mediating role in activation of enzymes and in this current situation of pandemic consumption of pumpkin can promote antioxidation in the living body thus restricting the attack of viral diseases (Hussain *et al*., 2021).

CONCLUSION

The results of this study shows that methanol extracts of pumpkin leaf and fruit are rich in carbohydrate and protein and could be a good source of carbohydrate and protein for people living in rural areas as well as for animals. This indicates that the plant is a good source of energy to both humans and animals. Pumpkin leaf and fruit methanol extracts possess numerous phytoconstituents which support their use in ethnomedicine. The methanol extract of pumpkin leaf and fruit exhibited significant antioxidant activity as revealed in the FRAP and DPPH assays, which could be attributed to the presence of phytochemical contents, especially flavonoid and alkaloid contents. The supply of minerals is important for the optimal function of the body system which was demonstrated by the presence of minerals in both extracts. The consumption of pumpkin should be encouraged, especially the leaf and fruit.

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EFFECT OF BOILING, FRYING AND FERMENTATION ON THE NUTRIENT AND ANTI-NUTRIENT COMPOSITION OF SWEET CASSAVA (*Manihot esculenta*)

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ABSTRACT

Cassava (*Manihot esculenta*) is a vital staple food nourishing around 800 million individuals globally, serving as a substantial calorie source. Nevertheless, its nutritional composition undergoes influences from diverse processing methods, which concurrently impact the presence of anti-nutrients. The aim of this study was to access how different traditional processing methods affects the nutritional and anti-nutritional composition of sweet cassava. Cassava roots were divided into four groups: raw, boiled, fried and fermented and processed accordingly. Proximate and anti-nutrient content of the roots were analyzed using standard methods. The result revealed that the carbohydrate content of boiled, fried and fermented roots were fairly improved (30.02±0.01%, 34.96±0.01%, 33.75±0.01% respectively) as compared to raw roots (27.98±0.01). All processing methods notably increased the protein content, with the most substantial increase seen in the fermented roots (3.26±0.01%). Frying effectively reduced moisture content (38.4±0.06%) compared to other processing methods, thereby increasing the shelf life. Fermentation and boiling were most effective in reducing the cyanide levels (0.34 mg/kg and 0.69 mg/kg respectively). This study highlights the importance of employing a suitable processing method to enhance nutrient retention and diminish anti-nutrient levels in cassava.

Keywords: *Manihot esculenta;* Traditional processing methods; Anti-nutrients; nutritional profile; Staple food

INTRODUCTION

Cassava (*Manihot esculenta*) is a drought-tolerant, staple food crop grown in tropical and subtropical areas ravaged with under-nutrition, making it a potentially valuable food source for developing countries such as Africa, Asia and Latin America. It is well known for its adaptability to a wide range of environmental conditions and unfertile soils as well as its capacity for high yields in about 90 days, making it an indispensable food security crop. The majority (70 %) of the world's cassava is produced in Nigeria, Brazil, Indonesia, Democratic Republic of Congo and Thailand (FAO, 2014). World annual cassava production has increased by approximately 100 million tonnes since 2000. This is driven by demand for cassava food products in Africa and for dried cassava and starch for use in livestock feed in Asia. Cassava is believed to represent the future of food security in some developing countries (Montagnac *et al*., 2009). In Nigeria and other African countries, cassava is commonly processed into *garri, fufu, tapioca* or consumed as boiled roots.

Cassava is a starchy root, with approximately 80–90% of its dry weight being carbohydrate. Fresh cassava roots contain 32%-35% carbohydrate (Shittu *et al*., 2023), and it is among the richest sources of energy from root crops. The fat content of cassava roots is less than 0.5% on a fresh weight basis, while the protein content is generally between 1% and 2%, expressed per fresh weight (Akinwale *et al*., 2022). Cassava is not particularly high in vitamins and minerals. Nevertheless, it provides small amounts of these micronutrients. Vitamin C, among other vitamins, is present in the fresh root relative to dried root, ranging from 15 mg to 45 mg/100 g, contributing to the antioxidant property (Olusanya *et al*., 2023). Calcium, phosphorus, iron, and magnesium are present in small quantities. Cassava is particularly rich in potassium, approximately 270 mg per fresh weight of 100 g (Oluwole *et al*., 2003). The presence of anti-nutrients such as cyanogenic glycosides, oxalates and phytates have been a major concern to the consumption of this staple, as these anti-nutrients can interfere with mineral absorption, as well as cause toxicity if not properly processed (Montagnac *et al*., 2009).

Despite the importance of this staple in food security, cassava's nutritional potential is frequently neglected due to significant nutrient losses caused by traditional processing methods. Boiling, frying, fermenting, and drying are common processing methods used to improve cassava edibility, palatability, shelf life, and safety. These processing methods are frequently applied in the production of *garri, fufu* and tapioca across Nigeria and other African countries (Okudoh *et al*., 2021). However, these activities can cause significant deterioration of critical nutrients such as vitamins, proteins, and minerals, all of which are necessary for human health (Okafor *et al*., 2020). Despite the use of traditional processing methods such as boiling, frying, fermenting, and drying, there is a dearth of information about their specific effects on

nutrient retention. During processing, nutrient can be lost by various means such as thermal and enzymatic degradation, leaching, oxidation and mechanical loss, with each method affecting different nutrients.

The necessity of improving processing methods to maintain cassava's nutritional content cannot be overstated, particularly in places where it is a key dietary component. Nutrient loss during processing not only reduces the nutritional value of food, but also has an influence on public health, especially in populations who rely heavily on cassava as a primary food source (Olawoye and Kolapo, 2018). As a result, there is an increased interest in inventing and marketing processing technologies that reduce nutritional losses while maintaining food safety and quality. Thus, it is important to investigate how different traditional processing methods affect the nutritional composition of cassava, with the goal of identifying methods that optimize nutrient retention and improve the dietary advantages of this important crop.

MATERIALS AND METHODS

Sample collection

Cassava roots were obtained from Kasuwa Gwari market in Minna, Niger state. The roots were identified by a botanist in the Department of Biological Sciences, Federal University of Technology, Minna, Niger State.

Preparation of raw and processed roots

The roots were peeled, washed, diced into small pieces and shared into four equal parts (A, B, C, D) ready for processing. Part A (raw sample) was grinded and stored in a container for further analysis. Three processing methods were employed: boiling, frying and fermentation. Part B was boiled for 25 minutes, allowed to cool and then stored in a container for further analysis. Part C was grinded, drained, folded into oval molds and fried in hot oil until it turned golden brown. Part D was mashed and stored in a container with minimal exchange of air for three days. On day three, the water content was drained out and the fermented root was stored in a container.

Proximate composition analyses

The moisture, fat, fibre and ash content of the raw and processed samples were determined by the method as described by Onwuka (2005). The moisture content was determined by oven drying method. Crude fat was determined by ether extract method using Soxhlet apparatus. The nitrogen was determined by micro Kjeldah method described by Onwuka (2005), and the nitrogen content was converted to protein content by multiplying by a factor of 6.25. Total carbohydrate content was estimated by 'difference', using the nitrogen-free method as described by AOAC (1990). This is calculated as weight by difference between 100 and the summation of other proximate parameters. All the proximate values were reported in percentage (%).

Determination of anti-nutrient content

Phytate content was determined using a modified indirect colorimetric method of Wheeler and Ferrel (1971). Samples were extracted with 3% trichloroacetic acid and filtered. Phytate was precipitated as ferric phytate by adding IM NaOH to the filtrate. Precipitate was dissolved in hot 3.2 M HNO and the absorbance was read at 480nm. The phytate percentage was calculated from the concentration of ferric iron assuming 4:6 iron:phosphorus molar ratio. The tannin content was determined by AOAC method, (1984). Samples were mixed with 50% methanol, covered with para film and incubated at 77-80 ^{0}C for 1hr. Extracts were filtered and mixed with Folin-Denis reagent and Na_2CO_3. The absorbance at 760 nm was recorded using a UV-spectrophotometer model 752, and tannin content was determined from a standard curve of tannic acid. Oxalate content was determined by permanganate titrimetric method as described by Oke (1966). Cyanide content was determined by alkaline picrate method according to Wang and Filled method as described by Onwuka (2005). Samples were extracted over-night with distilled water and then filtered. Filtrate were mixed with alkaline picrate and incubated in a water bath for 5mins. After color development, the absorbance was read at 490 nm. The cyanide content was extrapolated from a standard curve of KCN.

Statistical analysis

Data were analysed statistically using One-way ANOVA followed by a Duncan post hoc test, and values obtained are reported as mean ± SEM. Values of $p < 0.05$ are considered significant.

RESULTS

Proximate composition of cassava roots

The proximate composition of the raw and processed roots are shown in Table 1. Raw and boiled roots exhibited the highest moisture content, 66.42% and 65.42%, respectively, with no significant difference between them. Fried roots had the lowest moisture content, but the highest ash and fat content, which were significantly higher than that of all

other samples. Protein and fiber content were highest in the fermented roots. The carbohydrate content was well retained across all processing methods.

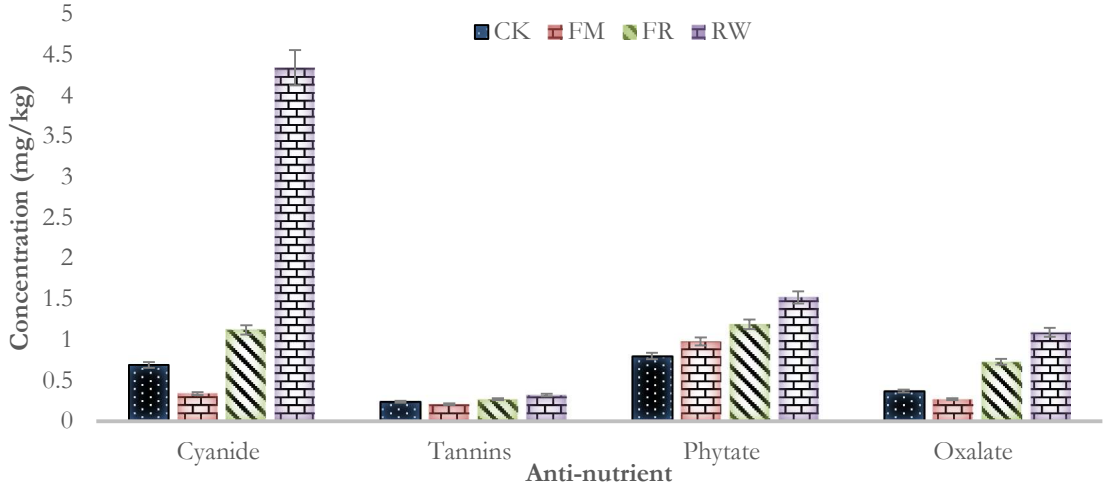
Table 1: Proximate Composition of Cassava Roots

Sample	Moisture (%)	Ash (%)	Fat (%)	Fiber (%)	Protein (%)	Carbohydrate (%)
BL	65.25±0.03[a]	1.02±0.01[a]	0.31±0.01[a]	2.13±0.03[a]	1.63±0.01[a]	30.02±0.01[a]
FM	55.75±0.03[b]	1.7±0.06[b]	1.16±0.03[b]	3.21±0.01[b]	3.26±0.01[b]	33.75±0.01[c]
FR	38.4±0.06[c]	2.07±0.01[c]	20.18±0.01[c]	2.03±0.03[a]	2.08±0.01[c]	34.96±0.01[c]
RW	66.42±0.36[a]	1.24±0.01[a]	0.4±0.00[a]	1.82±0.01[c]	1.09±0.01[d]	27.98±0.01[d]

The presented values represent the mean ± standard error of mean (SEM) from triplicate measurements. Significance among values within the same column indicated by different superscripts are considered significantly different at $p < 0.05$. BL: Boiled, FM: Fermented, FR: Fried, RW: Raw

Anti-nutrient content of cassava roots

The anti-nutrient content of the raw and processed roots are shown in Figure 1. The various processing methods considerably reduced the cyanide content of the roots as evident by the significantly high cyanide content of the raw roots. Tannin and oxalate content were slightly higher in the raw roots. Phytate content was highest in the raw roots and lowest in the boiled roots. The highest reduction in cyanide, tannin and oxalate content is seen in the fermented roots.



CK: Cooked, FM: Fermented, FR: Fried, RW: Raw

Figure 1: Anti-nutrient Content of Cassava Roots

DISCUSSION

The results obtained from the study revealed significant variations in both nutritional composition and anti-nutrient content of raw and processed roots, highlighting the impact of processing on quality and safety. The significant reduction in moisture content through frying aligns with findings by Zhang *et al.*, (2022), who reported that thermal processing substantially decreases water content in cassava, contributing to enhanced shelf stability. A relatively high moisture content indicates potential susceptibility to microbial spoilage (Otache *et al.*, 2023). All processing methods notably increased the protein content, with the most substantial increase seen in the fermented roots (3.26%). This protein enrichment through fermentation corroborates findings by (Amendola *et al.*, 2021), who attributed this increase to microbial protein synthesis during fermentation. The variation in carbohydrate content, ranging from 27.98% in raw roots to 34.96% in fried roots, reflects the concentration effect of moisture removal as explained by (Silva *et al.*, 2023). The increase in carbohydrate content from this study aligns with observations by Oladele *et al.* (2024).

All the processing methods employed decreased the anti-nutrient contents of the root. The substantial reduction in cyanide content through fermentation supports findings by Kumar *et al.* (2021), who emphasized the safety benefits of proper cassava processing. This underscores the necessity of processing before consumption, as stressed by Mensah *et al.* (2022). The consistent pattern of reduction in tannins, phytates, and oxalates across processing methods aligns with comprehensive studies by Liu *et al.* (2020) and Adebayo *et al.* (2023). Liu *et al.* (2020) suggested that combined processing techniques might be most effective for optimal anti-nutrient reduction.

CONCLUSION

The results obtained reveals that processing significantly affects the nutritional profile of cassava roots, as well as the anti-nutrient content, thus highlighting the importance of processing before consumption. Moisture content decreased significantly with processing, indicating the potential for stability and a longer shelf-life of processed products. All processing methods significantly enhanced the nutritional composition and effectively decreased the anti-nutrient content, contributing to increased bioavailability of micronutrients. Raw cassava roots consistently showed the highest levels of all anti-nutrients, emphasizing the necessity of processing before consumption. Each processing method offers distinct nutritional advantages and trade-offs. Thus, different processing methods can be employed, as well as combined, based on desired nutritional outcomes. These findings could lead to better nutritional outcomes and increased food security in cassava-dependent communities, and help agencies implement more effective agricultural and nutritional policies.

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A REVIEW OF PLANT-DERIVED ANTIMICROBIALS AS NATURAL SOLUTIONS TO ANTIBIOTIC RESISTANCE AND MICROBIAL CONTAMINATION

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ABSTRACT
Antibiotic resistance and microbial contamination pose significant threats to public health, food safety, and environmental sustainability. The overuse and misuse of synthetic antibiotics have led to the emergence of multidrug-resistant pathogens, necessitating alternative antimicrobial strategies. Plant-derived antimicrobials, including essential oils, phenolics, alkaloids, and flavonoids, have gained increasing attention due to their broad-spectrum antimicrobial properties, low toxicity, and potential synergistic effects. This review explores the mechanisms of action, sources, and applications of plant-based antimicrobials in addressing antibiotic resistance and microbial contamination. Furthermore, it highlights the challenges associated with their use and potential strategies for enhancing their efficacy in various sectors, including medicine, food preservation, and agriculture.

Keywords: Plant-based antimicrobials, antibiotic resistance, microbial contamination, essential oils, phenolics, food safety.

INTRODUCTION
The rise of antibiotic-resistant bacteria has significantly exacerbated global health crises, a concern underscored by the World Health Organization (WHO) labelling antimicrobial resistance (AMR) as one of the top ten threats to public health (Angelini, 2024). As traditional antibiotics lose efficacy due to widespread resistance mechanisms among pathogens, alternative strategies are urgently needed to combat these resistant strains. Notably, one promising avenue involves exploring plant-derived antimicrobials, which possess diverse bioactive compounds with significant antibacterial, antifungal, and antiviral properties. Recent research has demonstrated that various plant extracts exhibit remarkable antimicrobial potential. For example, studies have shown that specific plant extracts from traditional medicinal sources can effectively inhibit pathogenic microorganisms such as *Escherichia coli* and *Staphylococcus aureus* (Gadisa and Tadesse, 2021). This supports findings by Gonelimali *et al.* (2018) which articulated a direct correlation between the concentration of plant extracts and their antimicrobial activities against pathogens, suggesting the potential utility of these extracts in combating foodborne pathogens and spoilage organisms (Gonelimali *et al.*, 2018). Moreover, Sadgrove *et al.* (2020) confirmed that phytochemical constituents like flavonoids and alkaloids are crucial in the antimicrobial efficacy of many plant species, which aligns with findings that highlight mechanisms of action involving disruption of microbial cell membranes or inhibiting critical cellular pathways (Sadgrove *et al.*, 2020; Arip *et al.*, 2022). The application of nanotechnology further enhances the utility of plant derivatives in medical settings. For instance, Qanash *et al.* (2023) explored chitosan nanoparticles loaded with extracts from *Artemisia judaica*, demonstrating that such formulations could inhibit both microbial growth and cancer cell proliferation, thereby showcasing their multifaceted therapeutic potential. Additionally, the extract of *Solidago graminifolia* has been identified as an excellent source of polyphenolic compounds that exert both antioxidant and antimicrobial activities, reinforcing the idea that plant-derived substances can provide dual health benefits (Toiu *et al.*, 2019). The mechanisms by which plant-derived antimicrobials confer their action are varied. They can interfere with bacterial cell wall synthesis, disrupt membrane integrity, and inhibit nucleic acid or protein synthesis, which are pivotal in combating multiresistant strains (Chandra *et al.*, 2017; Khameneh *et al.*, 2019). Recent research aligns these mechanisms with specific compounds, such as isoflavones, which prove effective against antibiotic-resistant strains like methicillin-resistant *Staphylococcus aureus* (MRSA) (Sadgrove *et al.*, 2020). This illustrates a critical advantage of plant-derived antimicrobials: their potential to evade traditional resistance pathways that bacteria employ against synthetic antibiotics (Angelini, 2024; Huang *et al.*, 2022). As the challenge of AMR escalates, the exploration of plant-derived antimicrobials not only presents an immediate solution to counteracting resistance but also opens pathways for innovative formulations and treatments. Given the efficacy of these natural

compounds and their diverse mechanisms of action, integrating plant-based antimicrobials into mainstream medicine could play a crucial role in addressing the global public health threat posed by antibiotic-resistant bacteria.

Plant extracts have shown significant antimicrobial activity against various pathogenic microorganisms, making them a promising alternative to synthetic preservatives and biocides. This activity is primarily attributed to the presence of phenolic compounds, which exhibit both antioxidant and antimicrobial properties (Oulahal, and Degraeve, 2022). Extracts and essential oils from this plant have shown notable antimicrobial properties against foodborne pathogens (Sabo and Knezevic, 2019).

Methanol extracts from this plant, when used to synthesize zinc sulfide nanoparticles, exhibited both antimicrobial and photocatalytic degradation properties (Mani *et al.,* 2018). Basically, plant extracts, particularly those rich in phenolics and essential oils, offer a viable alternative to synthetic antimicrobials. However, further research is needed to optimize their application in food preservation and biofilm control, addressing challenges related to activity reduction in complex matrices and improving methodological approaches for accurate assessment (Oulahal and Degraeve, 2022; Eloff, 2019; Sabo and Knezevic, 2019)

DISCUSSION

Plant-derived antimicrobials are increasingly recognized for their potential to combat drug-resistant pathogens. These compounds, predominantly secondary metabolites, are produced by plants as defence mechanisms against microbial infections. Their classification typically includes essential oils, phenolic compounds, alkaloids, flavonoids, saponins, and terpenoids, each exhibiting unique modes of action against a variety of microorganisms.

Essential Oils are volatile compounds extracted from aromatic plants, and they have demonstrated significant antimicrobial activities. For example, components such as carvacrol and thymol, commonly found in essential oils from plants like thyme and oregano, are known to disrupt bacterial cell membranes, leading to cellular lysis and death (Pilau *et al*., 2011). Essential oils containing these compounds have been effective at low concentrations, inhibiting a broad spectrum of pathogens (Pilau *et al*., 2011). The antimicrobial actions are not strictly limited to bacteria; for instance, carvacrol has shown efficacy against viruses as well (Pilau *et al*., 2011).

Phenolic Compounds such as tannins and flavonoids play a crucial role in the antimicrobial properties of plant extracts. Tannins, for instance, have been shown to inhibit the metabolic processes of pathogens by disrupting cell walls and altering enzymatic functions (Štumpf *et al*., 2020). Compounds like curcumin, found in turmeric, exhibit multi-target activities against various bacteria, including resistant strains like Staphylococcus aureus and Escherichia coli (Adeyemı *et al*., 2020). The synergistic action of these phenolic compounds can also extend their efficacy to prevent biofilm formation, which is critical in treating persistent infections (Mahboubi *et al*., 2012).

Alkaloids such as berberine exhibit powerful antimicrobial properties, particularly by interfering with DNA replication and protein synthesis within bacterial cells (Adeyemı *et al*., 2020). Berberine, derived from plants like *Berberis*, has shown significant effectiveness against both Gram-positive and Gram-negative bacteria (Prasad *et al*., 2019). This mode of action highlights the versatile nature of plant-derived compounds in targeting essential cellular processes in pathogens.

Flavonoids, including quercetin and kaempferol, are recognized for their role in modulating microbial resistance mechanisms. They inhibit bacterial efflux pumps, enhancing the efficacy of antibiotics while reducing biofilm formation (Lee *et al*., 2011). Studies show that flavonoids can induce oxidative stress in bacterial cells, leading to cell death (Libério *et al*., 2011). This multifaceted approach not only aids in controlling current infections but also serves as a potential strategy against emerging resistant strains.

Saponins and Terpenoids contribute to the antimicrobial action through mechanisms such as cell membrane disruption and metabolic interference. Saponins have been shown to disrupt the integrity of microbial membranes, leading to the leakage of vital intracellular components (Zige *et al*., 2013). Similarly, terpenoids can modulate metabolic processes essential for bacterial survival, thereby augmenting their antimicrobial activity (Kha and Le, 2020). In summary, the diverse categories of plant-derived antimicrobials exhibit a range of mechanisms that highlight their potential as alternative therapies in combating microbial infections. As the search for new antimicrobial agents continues in light of rising drug resistance, these natural compounds offer a promising avenue for developing more effective treatment options.

Applications of Plant-Sourced Antimicrobials

Plant-sourced antimicrobials are increasingly recognized for their potential applications across various sectors, including medicine and pharmaceuticals, food preservation, agriculture, and environmental sanitation. This synthesis will explore these applications thoroughly, backed by relevant literature.

Medicine and Pharmaceuticals

Plant-derived antimicrobials exhibit remarkable prospects as alternative or complementary therapies to conventional antibiotics, particularly against antibiotic-resistant pathogens. Antimicrobial peptides (AMPs) from plants are a key focus in this arena. These peptides possess a diverse range of antimicrobial actions, capable of targeting various pathogens including bacteria, fungi, and viruses (Kuddus *et al.*, 2016). Significant research highlights the advantages of nanoparticles synthesized from plants—like silver nanoparticles (AgNPs)—which leverage plant extracts' bioactive compounds to enhance antimicrobial efficacy (Pereira *et al.*, 2024). The nanoparticles not only boost the activity of the constituents from which they are derived but also possess intrinsic antimicrobial properties, thus serving dual functions in therapeutic interventions (Pacyga *et al.*, 2025).

Food Preservation

Foodborne pathogens such as *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* pose significant health risks and economic challenges related to food spoilage. Plant antimicrobials, including essential oils and plant extracts, have been effectively integrated into food packaging and natural preservatives to ensure food safety and extend shelf life (Lu *et al.*, 2018; Awad *et al.*, 2022). For instance, the use of Nano emulsions containing essential oils has been shown to improve antimicrobial activity by enhancing the interaction of these oils with microbial membranes, leading to effective pathogen inhibition (Liang *et al.*, 2012). Additionally, efforts to incorporate antimicrobial agents from fermented plant substrates offer a promising strategy in the food industry where consumers demand natural preservatives (Saadoun *et al.*, 2022).

Agriculture and Livestock Production

The integration of plant-based antimicrobials as feed additives in livestock management is gaining traction. These substances not only serve to reduce reliance on antibiotic interventions but also bolster animal health and productivity (Lei *et al.*, 2018). Evidence indicates that essential oils can positively influence ruminal fermentation and nutrient digestibility in ruminants, effectively replacing traditional antibiotics (Lin *et al.*, 2013). The ongoing development of natural antioxidants from plant sources highlights their critical role in promoting sustainable agricultural practices while ensuring animal welfare (Lei *et al.*, 2018).

Environmental and Water Treatment

The application of plant-derived antimicrobials in environmental decontamination and water treatment showcases their versatility. Utilizing plant extracts in wastewater treatment can significantly reduce microbial loads, thereby improving water quality. This approach aligns with eco-friendly practices as these natural compounds typically demonstrate effective antimicrobial properties while minimizing chemical pollution (Patra and Baek, 2017; , Jiang *et al.*, 2023). The ability of plant extracts to stabilize nanoparticles further enhances their use in tackling persistent environmental contaminants (Pereira *et al.*, 2024).

Challenges and Future Perspectives

Despite these promising applications, the path to widespread use of plant-derived antimicrobials is fraught with challenges. Stability and bioavailability of these compounds are significant concerns, as many are sensitive to environmental conditions, which can lead to reduced effectiveness (Trinh *et al.*, 2022). Furthermore, the variability in plant composition due to environmental factors complicates standardization and dosage formulation (Coronas *et al.*, 2024). To address these issues, there is a growing interest in synergistic formulations that combine plant antimicrobials with traditional antibiotics or other carriers to improve efficacy and combat resistance mechanisms (Patra and Baek, 2017). Finally, the regulatory framework surrounding plant antimicrobials remains underdeveloped, posing challenges for commercialization (Saadoun *et al.*, 2022).In conclusion, plant-sourced antimicrobials represent a vital area of study with application potential in medicine, food preservation, agriculture, and environmental management. Continued research must address the challenges of stability, standardization, and regulatory approval to harness their full potential effectively.

Conclusion

Plant-derived antimicrobials offer promising natural solutions to antibiotic resistance and microbial contamination. Their diverse mechanisms of action, broad-spectrum activity, and potential for sustainable applications make them

valuable alternatives to conventional antibiotics. However, further research is needed to optimize their formulations, enhance stability, and develop cost-effective production methods. By integrating plant antimicrobials into medicine, food safety, and agriculture, we can pave the way for innovative strategies in combating microbial threats.

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EVALUATION OF SOME HAEMATOLOGICAL AND SERUM PARAMETERS OF BROILER CHICKENS ADMINISTERED PROBIOTICS (*LACTOBACILLUS FERMENTUM)* IN DRINKING WATER

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ABSTRACT

The experiment was conducted to examine the effect of probiotics (Lactobacillus fermentum) administered in water on haematological and serum biochemical parameters of cobb 500 broiler chickens. One hundred-day old chicks were randomly divided into five experimental groups and were replicated twice with ten birds per replicate, the experiment was laid out in a completely randomized design (CRD), the birds were fed with five probiotics diets supplements level inclusion of 0g, 7.5g, 15g, 22.5g and 30g in their water. Blood samples were collected from the birds' jugular vein at slaughter. The blood was received in a 10ml test tube containing EDTA (Ethylene Diamine Tetra acetic Acid). Haematological parameters measured were Haemoglobin, Packed Cell Volume, Mean Cell Volume, Mean Cell Haemoglobin, Mean Cell Haemoglobin Concentration, Red Blood Cell Count and Total White Blood Cell and The Serum biochemical constituents measured were Total bilirubin, Conjugated bilirubin, Alkaline phosphate, Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvic Transaminase (SGPT) and Total protein and Albumen all were subjected to ANOVA at 95% confidence level ($P < 0.05$) Treatment means were separated using the Duncan's multiple range test. The Haematological parameters had significant differences in all the parameters except in MCH, MCHC and TWBC. The birds fed with the test ingredient had higher ($P<0.05$) Hb, PCV, MCV and RBC value than birds in the control group. The Serum biochemical analysis showed significant differences for all the measured parameters except for Total protein and albumin. The birds on treatment 1 had higher ($P<0.05$) Alkaline value than birds fed with the test ingredient on T_2, T_4, T_3 and T_5 respectively. In conclusion, the present study showed that Lactobacillus fermentum treatment had effect ($P<0.05$) on all the haematological and serum biochemical parameters, except the Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), Total White Blood Cell Count (TWBC) in haematology and Total protein and albumin in serum biochemical parameters. For improve chickens blood haematology and serum biochemistry, 30g/4 litres of water is recommended as it gave better result on haematological and serum biochemical parameters.

Keywords: Probiotics, *Lactobacillus fermentum*, haematology, serum biochemistry.

INTRODUCTION

The poultry industry is increasingly focused on the role of probiotics in enhancing bird growth rates and carcass composition. Probiotics, live microorganisms that confer health benefits when administered in adequate amounts, are gaining recognition as a viable alternative to antibiotics in poultry production. While studies like those by Katoch *et al.* (1996) and Altaf-ur-Rahman *et al.* (2009) have highlighted the potential of various probiotics, many of these options are imported and costly, creating a need to explore readily available, locally sourced alternatives.

A critical issue facing the industry is the widespread use of antibiotics as growth promoters, which has led to significant concerns. Antibiotic residues in poultry products, such as meat and eggs, pose risks to human and animal health and are strongly linked to the escalating problem of bacterial resistance (Donoghue, 2003). With the European Union's ban on sub-therapeutic antibiotic use and similar considerations in the United States, the search for effective alternatives is paramount.

This urgency is further driven by growing consumer concerns about antibiotic residues and the rising demand for antibiotic-free poultry. Exploring alternative supplements, particularly locally sourced probiotics, becomes crucial due to their potential health-promoting effects. These benefits can include enhanced growth, improved egg quality, a strengthened immune system, and better overall health status in chickens. Therefore, this study is firmly grounded in the need for sustainable, safe, and cost-effective replacements for antibiotics.

A practical method for probiotic delivery is through drinking water, as highlighted by Ghadban (2002). Although challenges exist, like ensuring even distribution and maintaining the viability of anaerobic organisms in chlorinated water, this approach offers efficiency. Ghadban (1998) investigated various competitive exclusion techniques, including drinking water administration, and demonstrated positive results such as increased weight gain, reduced mortality rates, and improved feed conversion ratios.

Previous research further supports the potential impact of probiotics. Supplementation has been shown to influence haematological and serum biochemical parameters. For example, Siadati *et al.* (2017) observed significant effects of commercial and native probiotics on serum glucose, total protein, and other parameters in Japanese quails. Similarly, Oleforuh-Okoleh *et al.* (2015) found improvements in haemoglobin concentration and packed cell volume in broilers supplemented with aqueous extracts of ginger and garlic. These findings underscore the potential of probiotics, and specifically, this study will investigate the effects of *Lactobacillus fermentum* administered through drinking water on the physiological status of broiler chickens.

MATERIALS AND METHODS

Experimental Site and Animals

The research was conducted at the Poultry Unit of the Department of Animal Production Teaching and Research Farm, Federal University of Technology, Minna, Niger State, Nigeria. One hundred (100) day-old Cobb 500 broiler chicks were obtained from Olam Integrated Farm Nigeria Limited, located in Kaduna, Kaduna State, Nigeria. The chicks were weighed upon arrival and randomly assigned to the treatment groups.

Source of Probiotics and Experimental Design

The probiotic used in this study was *Lactobacillus fermentum*, purchased from Emmanuel Agrolife Benefits Enterprises in Lokoja, Kogi State, Nigeria. The Completely Randomized Design (CRD) was used for the experiment. The chicks were randomly divided into five treatment groups, each with two replicates. Each replicate consisted of ten birds, resulting in a total of twenty birds per treatment.

Treatment Groups and Administration

The five treatment groups were as follows:

- Treatment 1 (T1): Control group, had no probiotic supplementation.
- Treatment 2 (T2): *Lactobacillus fermentum* at 7.5g/4 litres of drinking water.
- Treatment 3 (T3): *Lactobacillus fermentum* at 15g/4 litres of drinking water.
- Treatment 4 (T4): *Lactobacillus fermentum* at 22.5g/4 litres of drinking water.
- Treatment 5 (T5): *Lactobacillus fermentum* at 30g/4 litres of drinking water.

The probiotic was administered daily through the drinking water, ensuring fresh solutions were prepared each day.

Bird Management and Housing

The broiler chicks were raised in a deep litter system. Routine management and standard vaccination programme schedules were strictly followed.

Data Collection and Analysis

At 56 days of age (slaughter age), blood samples were collected from the jugular vein of each bird. The blood was collected into 10ml test tubes containing EDTA (Ethylene Diamine Tetra acetic Acid) as an anticoagulant. Haematological parameters, including Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), Red Blood Cell Count (RBC), and Total White Blood Cell Count (TWBC), were determined using a Beckman Coulter ACT diff Haematology Analyzer (Beckman-Coulter, USA).

Serum biochemical constituents were also analysed. These included Total Bilirubin, Conjugated Bilirubin, Alkaline Phosphatase, Total Protein, Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvic Transaminase (SGPT), and Albumin.

The collected data were subjected to Analysis of Variance (ANOVA) using SPSS Package version 16.0 (2007). A confidence level of 95% ($P<0.05$) was used to determine statistical significance. Duncan's Multiple Range Test was employed to separate the treatment means where significant differences were observed.

RESULTS

Effect of *Lactobacillus fermentum* on Haematological Parameters

The haematological responses of broiler chickens to *Lactobacillus fermentum* administered in their drinking water are presented in Table 1.0. The results indicated that *Lactobacillus fermentum* treatment had a statistically significant effect ($P<0.05$) on Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Cell Volume (MCV), and Red Blood Cell Count (RBC). However, the treatment did not significantly affect ($P>0.05$) Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), or Total White Blood Cell Count (TWBC).

Specifically, chickens in T5 (30g/4L) exhibited significantly higher ($P<0.05$) Hb and PCV values compared to all other treatments. Chickens in T2 and T3 had comparable Hb values ($P>0.05$), but these were significantly higher ($P<0.05$) than those in T4 and T1. T4 birds had significantly higher Hb than T1 (control). Similarly, for PCV, T5 had the highest values, while T2, T3, and T4 were comparable but significantly higher than T1.

Regarding MCV, chickens in T2 had significantly higher ($P<0.05$) values than the other treatments. T3 and T4 were comparable, but significantly higher than T1. Interestingly, T1 had significantly higher MCV than T5. For RBC, T5 again showed significantly higher ($P<0.05$) values than all other treatments. T2, T3, and T4 had comparable RBC values, all significantly higher than T1.

Table 1: Haematological Parameters of Broiler Chickens Administered Probiotics (*Lactobacillus fermentum*) in their Water

Parameters	TREATMENT					SEM	P-value	Normal ranges
	T_1	T_2	T_3	T_4	T_5			
PCV (%)	30.00^{c}	35.00^{b}	35.00^{b}	34.00^{b}	37.00^{a}	0.656	0.00	35.90 - 41.00
Hb (g/dl)	10.70^{d}	11.70^{b}	11.70^{b}	11.40^{c}	12.30^{a}	0.141	0.00	11.60 – 13.68
RBC ($\times 10^{12}$/L)	3.9^{c}	4.10^{b}	4.20^{b}	4.10^{b}	4.60^{a}	6.56	0.00	4.21 – 4.84
TWBC($\times 10^{9}$/L)	1.16	1.20	3.75	1.22	1.30	6.06	0.66	4.07 – 4.32
MCV (Fl)	77.00^{c}	85.00^{a}	83.00^{b}	83.00^{b}	67.00^{d}	1.770	0.00	81.60 – 89.10
MCH (pg)	27.00	29.00	29.00	28.00	27.00	0.324	0.07	27.20 – 28.90
MCHC (g/dl)	28.00	29.00	30.00	30.00	30.00	0.306	0.12	32.41 – 33.37

Values across row with different superscripts are significantly different at $p < 0.05$.

Key: HB: Haemoglobin, PCV: Packed Cell Volume, MCV: Mean Cell Volume, MCH: Mean Cell Haemoglobin, MCHC: Mean Cell Haemoglobin Concentration, RBC: Red Blood Cell Count, TWBC: Total White Blood Cell Count, SEM: Standard Error Mean, P-value: Significant Value, T_1 – *Lactobacillus fermentum* at 0g (control) inclusion level, T_2: *Lactobacillus fermentum* at 7.5g/4litres inclusion level, T_3: *Lactobacillus fermentum* at 15g/4litres inclusion level, T_4: *Lactobacillus fermentum* at 22.5g/4litres inclusion level, T_5: *Lactobacillus fermentum* at 30g/4litres inclusion level.

Effect of *Lactobacillus fermentum* on Serum Biochemical Parameters

The serum biochemical responses of the broiler chickens are summarized in Table 2.0. The results showed a significant effect ($P<0.05$) of *Lactobacillus fermentum* treatment on Total Bilirubin, Conjugated Bilirubin, Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase, and Serum Glutamate Pyruvic Transaminase (SGPT). However, Total Protein and Albumin levels were not significantly affected ($P>0.05$) by the treatments.

Total Bilirubin was highest in T4, significantly different from all other treatments. T3 had higher Total Bilirubin than T1, which in turn was higher than T2. T2 had higher Total Bilirubin than T5. For Conjugated Bilirubin, T3 and T4 had significantly higher values than all other treatments and were comparable to each other. Alkaline Phosphatase was highest in T1, significantly different from the rest. T2 and T4 had comparable Alkaline Phosphatase levels, higher than T3, and T3 was higher than T5.

SGOT was highest in T5, significantly different from the rest. T4 had higher SGOT than T2, and T2 was higher than T3. T3, however, had higher SGOT than T1. SGPT was also highest in T5. T3 had higher SGPT than T2, and T2 had higher values than both T1 and T4, which were comparable.

Table 2: Serum Biochemical Parameters of Broiler Chickens Administered *Lactobacillus fermentum* Probiotics in their Water

	TREATMENT						
Parameters	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**SEM**	**P-value**
T. Bilirubin (umol/l)	5.20^{c}	4.90^{d}	5.90^{b}	6.40^{a}	4.60^{e}	0.178	0.00
C. Bilirubin (umol/l)	3.00^{b}	3.00^{b}	3.60^{a}	3.50^{a}	2.90^{b}	0.080	0.00
Alkaline P. (ukat/l)	10.00^{a}	8.80^{b}	5.00^{c}	7.77^{b}	4.10^{d}	0.615	0.00
SGOT ($\times 10^2$/ u/l)	2.3^{e}	2.98^{c}	2.73^{d}	3.05^{b}	3.11^{a}	7.894	0.00
SGPT (u/l)	3.8^{d}	3.70^{c}	4.70^{b}	2.80^{d}	10.10^{a}	0.698	0.00
Total Protein (g/dl)	3.00	3.10	2.80	3.10	2.80	0.106	0.85
Albumin (dl)	1.30	1.20	1.40	1.40	1.30	0.030	0.16

Values across row with different superscripts are significantly different at $p < 0.05$.
Key: T. Bilirubin: Total bilirubin, C. Bilirubin: Conjugated bilirubin, Alkaline P.: Alkaline phosphate, SGOT: Serum Glutamate Oxaloacetate Transaminase, SGPT: Serum Glutamate Pyruvic Transaminase

DISCUSSION

The results of this study demonstrate that dietary supplementation with *Lactobacillus fermentum* through drinking water significantly influenced several hematological and serum biochemical parameters in broiler chickens. The observed improvements in Hb, PCV, MCV, and RBC in the treated groups, particularly in T5 (30g/4L), suggest a positive impact of *Lactobacillus fermentum* on the hematopoietic system. The increased levels of these parameters as the dosage increased indicates a dose-dependent response. This enhancement could be attributed to the probiotic's influence on nutrient absorption, particularly iron, which is crucial for haemoglobin synthesis, or a stimulatory effect on hematopoietic organs. These findings align with the general understanding of probiotics' role in improving haematological profiles, as noted in the present results.

The values for PCV, Hb, RBC, and MCV obtained in this study were generally within the normal ranges reported by Campbell (2013) for broiler chickens, although some variations were observed. Specifically, the MCV values were, and while within Campbell's broader range, higher MCV values, as seen in some treatments, *could* indicate macrocytic anemia. However, given the concurrent increases in RBC and Hb, this is less likely a pathological condition and more likely a physiological response to the probiotic. The absence of significant differences in MCH and MCHC between the control and treatment groups supports this, suggesting that the *size* of the red blood cells might have been influenced, but not the hemoglobin concentration within those cells. This is an important distinction. This aligns with the findings of Al-Saad *et al.* (2014), who observed significant differences in haematocrit values with probiotic treatment. It contrasts, however, with Saied *et al.* (2011), who reported no effect of dietary probiotics on PCV. These discrepancies highlight the variability in responses to different probiotic strains, dosages, and administration methods.

The significant alterations in serum biochemical parameters, specifically Total Bilirubin, Conjugated Bilirubin, SGOT, Alkaline Phosphatase, and SGPT, indicate that *Lactobacillus fermentum* influenced liver function. The varied responses across different treatments suggest a complex interaction between the probiotic and liver metabolism.

The reduction in SGPT activity in the probiotic-supplemented groups (except T5 which was highest) compared to the control is noteworthy. Lower SGPT levels are generally considered indicative of improved liver health. This aligns with the findings of Priya (2013), who reported that probiotics improved haematological parameters, including a reduction in SGPT. The result for SGPT shows that the highest dosage has the highest SGPT.

The lack of significant differences in Total Protein and Albumin levels between the treatments suggests that the probiotic did not significantly impact protein synthesis or overall nutritional status, at least within the parameters of this study. This is consistent with some studies, such as those by Dimcho *et al.* (2005) and Djouvinov *et al.* (2005), who found no effect of probiotics on total protein and albumin levels. However, it contradicts findings by Onunkwo *et al.* (2018), who observed significant differences in total protein and globulin in broilers fed diets with direct-fed microbes. These differences could be due to variations in the type of probiotic, dosage, and broiler chicken breed.

The results support the potential of this locally sourced probiotic as a health-promoting supplement in poultry production, contributing to the broader effort to find alternatives to antibiotics. Further research is warranted to explore the mechanisms underlying these effects and to optimize the dosage and administration protocols for maximum benefit.

CONCLUSION

Probiotics are a key focus in biotechnological research, shifting from a historical view of bacteria as harmful to recognizing their benefits in animal health, feed, and preservation. Research now emphasizes the symbiotic relationship between poultry and their gut microbiota. As probiotics offer a promising alternative to antibiotics without promoting resistance, this study investigated *Lactobacillus fermentum* in broiler chickens' drinking water. It significantly ($P<0.05$) altered most haematological and serum biochemical parameters, except MCH, MCHC, TWBC, Total Protein, and Albumin, which were unchanged.

RECOMMENDATION

Based on the study's findings, *Lactobacillus fermentum* can be safely administered to broiler chickens through their drinking water without adverse effects. To optimize improvements in blood haematology and serum biochemistry, a dosage of 30g *Lactobacillus fermentum* per 4 litres of water is recommended, as this concentration provided the best results. Further research should explore the effects of water-administered probiotics, including *Lactobacillus fermentum*, on haematological and serum biochemical parameters in other livestock species to expand our understanding of probiotic benefits in animal agriculture.

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INFLUENCE OF AQUEOUS TURMERIC (*CURCUMA LONGA*) EXTRACT ON THE BACTERIAL COUNT IN BROILER CHICKEN MEAT

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ABSTRACT
This study investigates the potential of turmeric (*Curcuma longa*) aqueous extract as a natural alternative to synthetic growth promoters in broiler chicken production, focusing on its impact on meat bacterial count. One hundred and sixty broiler chicks were divided into four groups receiving varying concentrations of turmeric extract in their drinking water (0, 1, 2, and 3 g/L). Bacterial counts in meat samples were analysed. While no statistically significant differences ($P>0.05$) in bacterial load were observed across treatment groups, a numerical trend showed decreasing bacterial counts with increasing turmeric concentrations, ranging from 6.20 CFU/g (control) to 5.20 CFU/g (3 g/L turmeric). Although not statistically significant, this trend suggests a potential antimicrobial effect, warranting further research with higher turmeric concentrations and longer experimental durations to fully explore its potential as a natural feed additive for improving gut health in broiler chickens.

Keywords: *Curcuma longa*, broiler chicken meat, antimicrobial activity

INTRODUCTION
Turmeric (*Curcuma longa*) has a long history of use in both human and animal medicine. It is recognized for its diverse properties, functioning as a nematocide, antibacterial, antifungal, antiprotozoal, antiviral, and antioxidant agent (Chattopadhyay *et al.*, 2004; Kiuchi *et al.*, 1993). Beyond these, it also exhibits immunomodulatory and hypocholesteremic effects (Ammon *et al.*, 1993; Anthony *et al.*, 1999; Chattopadhyay *et al.*, 2004). In recent years, the poultry industry has experienced a surge in interest in medicinal plants. This shift is largely driven by growing consumer concerns regarding synthetic additives and antibiotic residues in meat products (Kirkpinar *et al.*, 2014). Prior research has already demonstrated some of turmeric's positive impacts on broiler chickens, including improvements in growth performance, feed conversion ratio, antioxidant status, and immune response (Al-Sultan, 2003; Emadi and Kermanshahi, 2006; Arslan *et al.*, 2017; Hosseini-Vashan *et al.*, 2012).

While the global benefits of turmeric are well-established, consumer demand for leaner meat, specifically options with lower intra-muscular fat, is on the rise. (Yang et al., 2009; Yang et al., 2015). In Nigeria, various locally available species of turmeric are commonly used in traditional medicine for treating a range of diseases. Given this widespread local use, it's crucial to specifically assess the effects, both positive and negative, of these local turmeric varieties on the carcass traits and sensory evaluation of broiler chickens.

This research is fundamentally driven by the necessity to explore the potential of turmeric as a natural and readily available substitute for synthetic growth promoters in antibiotic-free poultry production systems. It seeks to provide valuable insights into how an aqueous extract of turmeric might influence broiler production, with a particular focus on its impact on the bacterial count present in the meat and on the overall quantitative phytochemical profile of the turmeric itself. Ultimately, this investigation will contribute to developing more scientifically grounded and sustainable practices within the poultry industry. Therefore, the core aim of this study is to rigorously examine the effect of an aqueous extract of *Curcuma longa* (turmeric) on the bacterial count found in broiler chicken meat.

MATERIALS AND METHODS
Experimental Site and Bird Management
The experimental work was conducted at the Poultry section of the Teaching and Research Farm, Department of Animal Production, School of Agriculture and Agricultural Technology, Federal University of Technology, Gidan Kwano Campus, Minna, Nigeria.

One hundred and sixty (160) day-old Ross 308 broiler chicks were randomly assigned to four treatment groups in a Completely Randomized Design (CRD). Each treatment group was replicated four times, with ten birds per replicate

(40 birds per treatment). The birds were housed in a poultry pen with two-sided openings for ventilation, using a deep litter system. Routine management and standard vaccination programme schedules were strictly followed.

Experimental Materials and Diet Formulation

The experimental test ingredient, turmeric, was procured from Kure ultra-modern market, Minna, Niger State. A single-phase feeding method was employed. The treatment groups were as follows:

- **Treatment 1 (T1):** 0 g turmeric/litre of water (Control)
- **Treatment 2 (T2):** 1 g turmeric/litre of water
- **Treatment 3 (T3):** 2 g turmeric/litre of water
- **Treatment 4 (T4):** 3 g turmeric/litre of water

Turmeric Extract Preparation

Fresh turmeric rhizomes were obtained from Kure ultra-modern market, Minna. The rhizomes were washed, sliced, and sun-dried for four days. The dried turmeric was then ground into a powder using a hammer mill. The turmeric aqueous extract was prepared by dissolving the weighed turmeric powder in water, stirring, allowing it to stand for 30 minutes, and then filtering it through a muslin cloth. The filtrate was collected and used as the aqueous extract, administered to the birds via their drinking water.

Bacterial Count Analysis

Total aerobic mesophilic counts (CFU/g x 10^5) were determined following the procedures of AOAC (2005). Dilutions of meat samples were plated on plate count agar and incubated aerobically at 37°C for 24 hours. After incubation, bacterial colonies were counted and averaged. The result was expressed as the total number of colonies multiplied by the dilution factor and inoculum volume, yielding colony-forming units per gram (CFU/g x 10^2).

Data Analysis

All data was analysed using a one-way analysis of variance (ANOVA) in SPSS software (version 23.0). Significant differences between treatment means were determined using the same software.

RESULTS

The analysis of bacterial counts in broiler chicken meat, following the administration of varying levels of turmeric aqueous extract, revealed no statistically significant differences (P>0.05) among the treatment groups. The bacterial loads ranged from 6.20 CFU/g in the control group (T1) to 5.20 CFU/g in the group receiving the highest concentration of turmeric extract (T4). While statistical significance was not achieved, a numerical trend of decreasing bacterial load was observed with increasing concentrations of turmeric aqueous extract. The specific bacterial counts for each treatment group, presented as colony-forming units per gram (CFU/g), are visually represented in Figure 1.

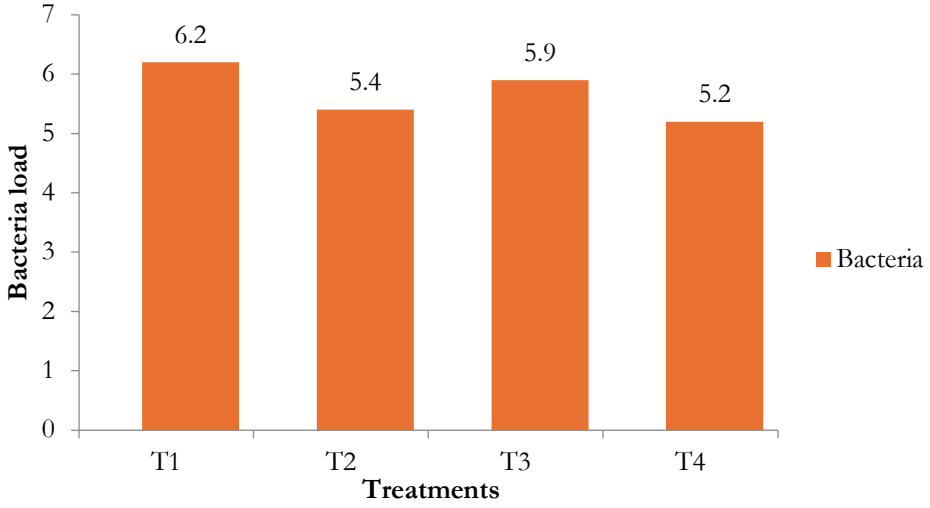

Figure 1: Bacterial Count of Broiler Chicken Administered Turmeric Aqueous Extract.

T1 = Control (administered 0 g of turmeric/litre of water); T2 = administered 1 g of turmeric/litre of water; T3 = administered 2 g of turmeric/litre of water; T4 = administered 3 g of turmeric/litre of water; CFU = Colony forming unit; SEM = Standard error of mean; P-value = Probability value; LS = Level of significance; NS = Not significant.

DISCUSSION

The results of this study, focusing on the bacterial count in broiler chickens administered varying concentrations of turmeric aqueous extract, revealed no statistically significant differences ($p>0.05$) across the treatment groups. As presented in Figure 1, the bacterial load, measured as colony-forming units per gram (CFU/g), ranged from 6.20 CFU/g in the control group (T1) to 5.20 CFU/g in the group receiving the highest concentration of turmeric extract (T4). While the absence of statistical significance might initially suggest a lack of effect, the consistent numerical decrease in bacterial load observed with increasing turmeric concentration warrants careful consideration.

The numerical trend, with bacterial counts decreasing from 6.20 CFU/g (T1) to 5.4 CFU/g (T2), 5.9CFU/g, then to 5.20 CFU/g (T4), strongly suggests a potential antimicrobial effect of the turmeric aqueous extract. This observation aligns with a growing body of literature highlighting the antimicrobial properties of turmeric and its bioactive components. Kumar *et al.* (2017) reported on the antimicrobial activity of turmeric against common foodborne pathogens, supporting the potential for turmeric to contribute to a reduction in bacterial load. Furthermore, Sarker *et al.* (2018) specifically demonstrated the antimicrobial effects of turmeric against poultry pathogens, including *E. coli* and *Salmonella*, two bacteria of significant concern in poultry production and food safety.

The active compound primarily responsible for turmeric's antimicrobial activity is curcumin. Tyagi *et al.* (2015) demonstrated that curcumin inhibits both bacterial growth and biofilm formation, a crucial mechanism by which bacteria establish themselves and resist antimicrobial agents. The observed numerical decrease in bacterial load in our study, while not statistically significant, could be attributed to the action of curcumin and other bioactive compounds present in the turmeric aqueous extract.

Several factors could explain why the observed trend did not reach statistical significance. One possibility is the dosage of turmeric aqueous extract used in the study. The concentrations (1 g/L, 2 g/L, and 3 g/L) might have been too low to elicit a statistically significant reduction in bacterial load. Future research should explore higher concentrations of turmeric extract to determine if a more pronounced and statistically significant effect can be achieved.

Another factor to consider is the duration of the experiment. The length of time the birds were exposed to the turmeric extract might have been insufficient to allow for a statistically significant change in bacterial load to manifest. A longer experimental period could potentially reveal a more substantial and statistically significant impact.

The method of administration, via drinking water, also warrants consideration. While convenient, this method might have resulted in variations in the actual intake of turmeric extract by individual birds, potentially contributing to the lack of statistical significance.

Despite the absence of statistical significance in this specific study, the findings contribute to the broader discussion surrounding the use of natural alternatives to antibiotics in poultry production. The numerical decrease in bacterial load, coupled with the existing literature on turmeric's antimicrobial properties, suggests that turmeric aqueous extract holds promise as a potential feed additive to promote gut health and potentially reduce the reliance on antibiotics. This is particularly relevant given the growing concerns about antibiotic resistance and the need for alternative strategies to manage bacterial populations in poultry (Lillehoj *et al.*, 2018). Further research, employing higher doses, longer experimental periods, and potentially different administration methods, is needed to fully elucidate the potential of turmeric aqueous extract as a natural antimicrobial agent in broiler chicken production.

CONCLUSION AND RECOMMENDATIONS

In this study, it was established that the inclusion of turmeric aqueous extract in the drinking water of broiler chickens, at the concentrations tested, did not significantly alter the bacterial count in the meat. However, a consistent numerical decrease in bacterial load was observed with increasing concentrations of the extract, suggesting a potential, albeit non-significant, antimicrobial effect.

Based on the findings of this study, although the tested levels of turmeric aqueous extract didn't statistically confirm its impact, the numerical trend encourages that furthur research should explore higher concentrations and longer experimental periods to fully understand the dose-response relationship.

It is therefore recommended that further research should be conducted, to explore higher concentration of Turmeric aqueous extract, this will help validate its potential as a natural feed additive for improving gut health and potentially reducing bacterial load in broiler chicken meat. This research should also investigate different period of administering the extract to chicken.

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ASSESSMENT OF THE ORGANOLEPTIC ATTRIBUTES OF BROILER CHICKENS FED VARYING LEVELS OF BOILED LEBBECK (ALBIZIA LEBBECK) SEED MEAL BASED DIETS

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ABSTRACT
This study assessed the influence of boiled *Albizia lebbeck* seed meal on the sensory attributes of broiler chicken meat. One hundred and sixty (160) day-old Ross-308 broiler chicks were randomly assigned to four treatment groups in a completely randomized design (CRD) with four replicates of ten (10) birds each. Treatment 1 served as the control (0% *Albizia lebbeck*), while Treatments 2, 3, and 4 included boiled lebbeck at 1.5%, 3.0%, and 4.5% inclusion levels, respectively, for seven weeks. Data were analysed using One-way ANOVA. Results indicated that lebbeck inclusion had no significant effect (P>0.05) on sensory parameters, including meat colour (6.67–7.00), juiciness (7.00), appearance (6.67–7.00), flavour (6.67–7.00), aroma (6.67–7.00), tenderness (7.33–7.67), and overall acceptability (7.33–7.00). The study suggests that lebbeck seed meal-based diets do not negatively impact the sensory qualities of broiler meat.

Keywords: *Albizia lebbeck,* broiler chickens, sensory evaluation

INTRODUCTION
Consumer perception plays a vital role in determining meat quality, which is often evaluated based on sensory characteristics such as taste, colour, juiciness, tenderness, and aroma (Schivazappa and Virgili, 2020). These sensory attributes, along with other quality metrics like water holding capacity, cooking loss, shear strength, and meat pH (Font-i-Furnols and Guerrero, 2014), influence consumer preference and shelf life. With increasing demand for healthy, lean, and low-fat meat options (John *et al*., 2016), broiler meat, known for its low transfat content (Greger, 2014), presents a favourable alternative to beef. The type of feed administered during broiler rearing significantly affects meat quality (Abd El-Hack *et al*., 2019). However, the high cost of feed, accounting for up to 70% of total production costs, remains a major challenge in broiler production (Abbas, 2013). Conventional feed resources are often inadequate, seasonal, and face competition from human consumption. This necessitates the exploration of alternative feedstuffs to reduce production costs and enhance the affordability of meat products (FAO, 2014).

Albizia lebbeck seed, also known as flea tree, fry wood, siris, woman tongue, or East Indian walnut, is a leguminous tree native to tropical and subtropical regions (Lamb *et al*., 2005). Traditionally, its leaves have been used to treat various ailments. Reported biological activities include antimicrobial, anti-inflammatory, antifungal, antispasmodic, antiviral, and neuroprotective properties. These diverse properties make lebbeck a potential bridge between food safety and livestock nutrition.

The increasing consumer preference for meat with lower intramuscular fat drives the need for nutritional strategies using accessible and cost-effective feed resources like lebbeck (Yang *et al*., 2009; Yang *et al*., 2015). *Albizia lebbeck* seed can replace soybean, groundnut cake, and maize, thereby lowering production costs. However, raw lebbeck seed contains antinutritional substances and toxic factors (Arif *et al*., 2017) that must be addressed through appropriate processing. Overcoming the challenge of protein insufficiency in animal feeds is essential, and the successful incorporation of lebbeck seeds in broiler chicken feeds could significantly contribute to solving this issue.

Lebbeck seeds offer a rich source of nutrients (Oyelere *et al*., 2016) and represent an unconventional feed resource that does not directly compete with human consumption, making it ideal for boosting poultry production. Lebbeck's anti-nutritional factors can be degraded by boiling with water.

MATERIALS AND METHODS
Location of Experimental Study
The experiment was conducted at the Poultry Unit of the Teaching and Research Farm of the Department of Animal Production, School of Agriculture and Agricultural Technology, Federal University of Technology, Minna, Niger State.

Source of Experimental Animals and Test Material

One hundred and sixty (160) day-old Ross-308 broiler chicks were obtained from Agri-Tech Ibadan, Oyo State, Nigeria. Seed pods of *Albizia lebbeck* were harvested from trees located within the premises of the Federal University of Technology, Minna, Niger State.

Experimental Design and Management of Birds

The 160-days-old Rose-308 broiler chicks were randomly distributed into four dietary treatment groups, each consisting of 40 birds. Each treatment was further divided into four replicates, with 10 birds per replicate. The experiment followed a completely randomized design (CRD). Standard vaccination protocols and prophylactic treatments were administered to the birds as needed throughout the duration of the study.

Experimental Diets

Four experimental diets were formulated to represent the four treatment groups. Diet 1 (T1) served as the control diet, containing 0% boiled *Albizia lebbeck*. Diet 2 (T2) consisted of 1.5% inclusion of boiled *Albizia lebbeck*. Diet 3 (T3) contained a 3.0% inclusion level of boiled *Albizia lebbeck*, while Diet 4 (T4) included 4.5% boiled *Albizia lebbeck*. All diets were formulated for single-phase feeding to meet the nutritional requirements of the broilers.

Evaluation of Organoleptic Attributes

The Keeton method was used to evaluate the organoleptic attribute of the meat samples. Breast meat samples were collected from randomly selected birds of each treatment group at the end of the experiment. The meat samples were then boiled in a pot containing 150ml of water for ten minutes, and one gramme of salt was added. The meat samples were cut into smaller pieces of about 3-5 grammes of about 1-2 cm thickness according to their treatments and replicates. A panel of trained assessors evaluated the cooked meat samples for tenderness, colour, juiciness, flavour, aroma, and overall acceptability using a 9-point hedonic scale developed by (Peryam *et al*., 2004). The grading system used was as follows: 9. Like extremely, 8. Like very much, 7. Like moderately, 6. Like slightly, 5. Neither dislike nor like, 4. Dislike slightly, 3. Dislike moderately, 2. Dislike very much, 1. Dislike extremely.

Data Analysis

Data collected on the organoleptic attributes were subjected to one-way analysis of variance (ANOVA) using SPSS (2017) statistical software. Where significant differences were observed, Duncan's multiple range test was applied to separate the means and determine the significance of the differences between treatment groups.

RESULTS

The impact of dietary inclusion of boiled *Albizia lebbeck* on the sensory attributes of broiler chicken meat was evaluated. The results of the sensory evaluation, encompassing parameters such as meat colour, juiciness, appearance, flavour, aroma, tenderness, and overall acceptability, are summarized in Table 1. The analysis revealed no statistically significant differences ($P>0.05$) between any of the treatment groups across all sensory parameters assessed. Specifically, meat colour scores ranged from 6.67 to 7.00, juiciness remained consistent at 7.00, appearance ranged from 6.67 to 7.00, flavour ranged from 6.67-7.00, aroma ranged from 6.67 to 7.00, tenderness ranged from 7.33-7.67 and overall acceptability ranged from 7.33-7.00. These findings indicate that the inclusion of boiled lebbeck in broiler diets, at the tested levels, did not significantly alter the sensory profile of the resulting chicken meat.

Table 1: Sensory Evaluation of Broiler Chickens Fed Varying Levels of Boiled lebbeck (*Albizia lebbeck*)

	Treatment						
Parameters	**T1**	**T2**	**T3**	**T4**	**SEM**	**P.V**	**LS**
Colour	7.00	6.67	7.00	7.00	0.08	0.441	NS
Juiciness	7.00	7.00	7.00	7.00	0.12	1.000	NS
Appearance	7.00	6.67	7.00	7.00	0.08	0.441	NS
Flavour	7.00	6.67	6.67	7.00	0.11	0.596	NS
Aroma	6.67	7.00	7.00	7.00	0.08	0.441	NS
Tenderness	7.67	7.33	7.67	7.67	0.15	0.859	NS
Overall Acceptability	7.67	7.33	7.33	7.67	0.15	0.802	NS

Keys: T1 = 0 % inclusion level of boiled Albizia lebbeck, T2 = 1.5 % inclusion level of boiled Albizia lebbeck, T3 = 3.0 % inclusion level of boiled Albizia lebbeck, T4 = 4.5 % inclusion level of boiled Albizia lebbeck, SEM = Standard Error of Mean, P.V = Probability Value, LS = Level of significance, NS = Not significant

DISCUSSION

The results of this study demonstrate that the inclusion of boiled *Albizia lebbeck* (*A.* lebbeck) seed meal in broiler diets did not significantly affect the sensory characteristics of the resulting chicken meat (P>0.05). Specifically, the panel of assessors found no discernible differences in colour, juiciness, appearance, flavour, aroma, tenderness, or overall acceptability across the treatment groups, as presented in Table 1. These findings align with previous research by Tsado *et al.* (2018), who also reported that dietary treatments did not significantly influence colour, tenderness, and overall acceptance in poultry.

The consistent meat colour scores, ranging from 6.67 to 7.00 across all treatments, suggest that lebbeck did not negatively impact the visual appeal of the meat. Meat appearance is a critical factor influencing consumer choice (Eldesouky *et al.*, 2015), as it often serves as the initial indicator of quality. Similarly, the uniform juiciness scores (7.00) indicate that *Albizia lebbeck* inclusion did not compromise the water-holding capacity or perceived moistness of the meat.

Flavour and aroma, both essential sensory attributes that drive appetite and overall eating experience, also remained unaffected by the dietary treatments. The similar flavour scores (6.67-7.00) and aroma scores (6.67-7.00) suggest that *Albizia lebbeck* did not introduce any undesirable tastes or odours to the chicken meat. The tenderness scores, ranging from 7.33 to 7.67, further confirm that *Albizia lebbeck* did not alter the textural properties of the meat.

Overall acceptability, a comprehensive measure of consumer satisfaction, exhibited no significant variation among the treatment groups, with scores ranging from 7.33 to 7.00. This finding underscores the potential of *Albizia lebbeck* as a feed ingredient without compromising the overall sensory quality of broiler meat.

A detailed assessment of meat quality through sensory evaluation is essential for informing producers, distributors, and consumers in making informed decisions about broiler breeds and meat products. The current study suggests that boiled *Albizia lebbeck* can be included in broiler diets without negatively affecting sensory attributes.

It is possible that the boiling process used to treat the *Albizia lebbeck* seed meal effectively mitigated any potential negative effects on sensory parameters. Boiling may have reduced the levels of antinutritional factors or volatile compounds that could have otherwise impacted the taste, odor, or texture of the meat. Further research may be warranted to investigate the effects of other processing methods or higher inclusion levels of lebbeck on sensory characteristics.

CONCLUSION

Based on the findings of this study, dietary supplementation with *Albizia lebbeck* seed meal, at the tested inclusion levels, did not exert any statistically significant influence on the sensory attributes of broiler chicken meat, including colour, juiciness, appearance, flavour, aroma, tenderness, and overall acceptability. Therefore, *Albizia lebbeck* appears to be a viable feed ingredient that does not negatively impact the palatability and consumer acceptance of broiler meat.

RECOMMENDATION

Considering the sensory evaluation results, a supplementation level of 1.5% *Albizia lebbeck* is suggested, given that this treatment group was perceived to be slightly superior to the control group (T1). To further optimize the utilization of *Albizia lebbeck* in broiler diets, future research should focus on exploring a wider range of inclusion levels and investigating various processing techniques aimed at enhancing the flavour, tenderness, and overall acceptability of the resulting broiler meat.

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EFFECT OF AFLATOXIN B_1 CONTAMINATION ON THE NUTRIENT COMPOSITION OF SOME CEREAL CROPS FROM AGROECOLOGICAL ZONES IN NIGERIA

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ABSTRACT

Aflatoxin B_1 contamination of cereals is a significant food safety concern due to its toxicity and impact on nutritional quality, particularly in regions with poor storage and handling practices. This study investigates fungal contamination, aflatoxin B_1 levels, and their effects on the nutrient composition of maize, rice, and sorghum from Guinea, Sudan, and Derived Savannah zones in Nigeria. A total of 27 samples were analyzed for colony-forming units (CFU/g), aflatoxin B_1 concentrations, and nutrient composition using standard protocols. Sorghum from the Derived Savannah recorded the highest aflatoxin B_1 concentration (81.47 ± 5.14 µg/kg), correlating negatively with carbohydrate content ($r = -0.995$, $p < 0.05$) and protein content ($r = -0.785$), reflecting significant nutrient depletion. Maize samples from the Sudan Savannah exhibited the highest fungal load (1.26×10^5 CFU/g) but moderate aflatoxin B_1 levels (16.03 ± 18.12 µg/kg). Despite lower fungal contamination, rice from the Guinea Savannah had elevated aflatoxin B_1 levels (37.20 ± 39.05 µg/kg), highlighting the ability of aflatoxin-producing fungi to thrive under specific conditions. Across all cereals, moisture content was positively correlated with aflatoxin B_1 concentrations ($r = 0.998$, $p < 0.05$), indicating that high humidity exacerbates contamination risks. Nutrient analysis revealed that aflatoxin B_1 significantly reduces carbohydrate and protein content, while having variable effects on fat, fiber, and ash content depending on the cereal and region. These findings underscore the urgent need for enhanced storage infrastructure, drying practices, and regulatory interventions to mitigate aflatoxin contamination, safeguard nutritional quality, and ensure food safety in Nigeria's cereal crops.

Keywords: Aflatoxin B_1, nutrient composition, fungal contamination, Guinea Savannah, Sudan Savannah, Derived Savannah, food safety.

INTRODUCTION

Fungi are prevalent plant pathogens and significant food spoilage agents (Makun *et al*., 2009). They can attack crops both in the field and during storage, thriving in various environmental conditions (Rouaa *et al*., 2021). Fungi produce mycotoxins — poisonous secondary metabolites—that contaminate food and feed, leading to reduced crop yield and quality, and causing substantial economic losses (Zain *et al*., 2011). Ingesting mycotoxin-contaminated grains poses serious health risks, as these toxins can be nephrotoxic, immunotoxic, teratogenic, and mutagenic, resulting in acute and chronic health issues (Bhat and Vasanthi, 2003).

Cereal products are particularly vulnerable to contamination by mycotoxin-producing fungi, especially from the *Aspergillus* genus (Frenich *et al*., 2009). Aflatoxins, particularly AFB_1, are highly toxic and classified as carcinogenic (IARC, 2016). Regulatory bodies like NAFDAC in Nigeria and the European Commission set maximum allowable mycotoxin levels to protect public health (NAFDAC, 2021; EC Regulation No. 1881/2006).

The economic and health risks associated with fungal and mycotoxin contamination, especially in developing countries, necessitate periodic monitoring to ensure food safety and compliance with regulations. This study investigates the fungal contamination profiles in maize, rice, and sorghum from various regions in Nigeria, as well as the concentrations of aflatoxins B_1, and their effects on the nutrient composition of these crops.

MATERIALS AND METHODS

Sampling location, collection and preparation

In the year 2024, visibly mouldy samples of maize, rice and sorghum were collected during the hot, dry season (February – April), from four local government areas: Ajingi, Guma, Baruten and Mokwa, in Kano, Benue, Kwara and Niger states respectively.

During this period, approximately 2 kg of farmed, stored, and marketed samples were collected from each location. The samples were carefully labeled, packaged in sterile polythene bags, and transported to the laboratory for analysis. Upon arrival at the laboratory, each sample was divided into two equal portions. One

portion was ground and further divided into two halves: one half was stored under appropriate conditions for subsequent mycotoxin analysis, while the other half was used immediately for fungal isolation studies.

A total of Twenty-seven (27) samples, at least 2kg each were collected; 21 sorghum and 6 rice from several towns in Northern Nigeria. Sorghum was collected from Ajingi, Kano (Derived savannah), Mokwa, Niger (South Guinea savannah), and Guma, Benue (Derived savannah). While Rice was collected from Baruten, Kwara (South Guinea savannah).

Isolation and Identification of Fungi

The mycological analytical procedure involving four steps according to the method of Makun *et al.* (2011) was used including fungal isolation on potato dextrose agar (PDA), subculturing on PDA, malt extract agar (MEA) and yeast extract agar (YEA), macro- and microscopic identification and finally, phylogenetics of fungi.

Primarily, each milled sample was subjected to a six-level serial dilution technique in which 1g was diluted in a 9-mL of sterile distilled water, vortexed, and subsequently, 1 mL of the suspension was transferred to a 9 mL of sterile distilled water, and vortexed, and so forth. 100µL of each suspension was inoculated on solid PDA containing 1 % Chloramphenicol in 90mm Petri dishes and incubated at 28 ± 2 °C for 3-5 days. Between the 3rd and 5th day of incubation, all colonies were counted and results presented as the number of fungal colonies per gram of sample calculated and expressed in colony-forming units per gram (cfu/g) as shown in Equation 1.

$$\text{CFU/g} = \frac{\text{Number of colonies X Reciprocal of the dilution factor}}{\text{plating volumes (mL)}} \times 100$$

Equation 1

The determination of each species of fungi was done using the keys of Klich and Pitt (1988), Klich (2002), and Nyongesa *et al.* (2015), for *Aspergillus spp.* And Pitt and Hocking (1997) for *Penicillium* and other genera. This was done by observing both after incubation in the Potato Dextrose Agar (PDA), the Malt Extract Agar (MEA) and in the Yeast Extract Agar (YEA) medium.

The fungal morphology was studied macroscopically by observing the colony features (color, shape, size and hyphae), and microscopically by a compound microscope with a digital camera using a lactophenol cotton blue-stained slide mounted with a small portion of the mycelium.

The summary of percentage incidence rate, frequency and relative density of the isolated fungi species from maize samples were calculated using Equation 2, 3 and 4 respectively.

$$\text{Incidence rate (\%)} = \frac{\text{Number of Isolates}}{\text{Total Number of Isolates}} \times 100$$

Equation 2

$$\text{Frequency (\%)} = \frac{\text{Number of Isolates}}{\text{Total Samples}} \times 100$$

Equation 3

$$\text{Incidence rate (\%)} = \frac{\text{Number of Isolates}}{\text{Total Incidence of Isolates}} \times 100$$

Equation 4

Extraction and Identification of Mycotoxins

1. Aflatoxin quantification using indirect enzyme-linked immunoassay (ELISA).

Aflatoxin quantification using an Indirect Enzyme-Linked Immunosorbent Assay (ELISA) begins by coating an ELISA plate with 150 µl of a diluted aflatoxin-BSA conjugate, followed by incubation at 37°C for 1 hour with shaking (Tang *et al*., 2019). After discarding the contents, the plate was washed three times with PBS-Tween, and 150 µl of 0.2% BSA was added to each well for a 30-minute incubation at the same temperature to block non-specific binding sites (Kim *et al*., 2021). The aflatoxin standard was prepared by diluting 1.5 µl of the standard in a methanol-PBST mixture, with serial dilutions made using the same diluent. Samples were prepared by diluting 20 µl of the sample in 180 µl of 0.2% BSA, and 100 µl of both standards and samples was dispensed into the wells, followed by the addition of 50 µl of antiserum (Li *et al*., 2022). The plate underwent a 1-hour incubation at 37°C and was washed again. Then, 150 µl of goat anti-rabbit antibody was added, and the plate was incubated for another hour before further washing. A substrate solution of para-nitrophenyl phosphate was added, and after a 30-minute incubation, the absorbance was measured at 405 nm using an ELISA plate reader to determine the aflatoxin concentration (Zhang & Zhao, 2020).

2. Aflatoxin quantification using High-Performance Liquid Chromatography (HPLC)

The principle of aflatoxin quantification using High-Performance Liquid Chromatography (HPLC) involves separating, detecting, and quantifying aflatoxins based on their unique chemical properties. Initially, samples were subjected to extraction procedures using solvents 100% methanol to isolate aflatoxins (Agbetiameh *et al*., 2021). The extract was then purified, through solid-phase extraction (SPE), to remove impurities and concentrate the aflatoxins. HPLC relies on a stationary

phase (a column) and a mobile phase, typically consisting of water and organic solvents, to separate compounds as they pass through the column at different rates (Kumar *et al*., 2020).

In the HPLC aflatoxin quantification, a fluorescence detector was employed due to aflatoxins' natural fluorescence. When exposed to ultraviolet light, aflatoxins emit fluorescence, allowing precise detection at low concentrations (Rahmani *et al*., 2022). Quantification is achieved by comparing the sample's peak area or height against a calibration curve created using known aflatoxin standards. This method ensures accurate and sensitive aflatoxin detection, with detection limits in the low parts-per-billion (ppb) range (Williams *et al*., 2019).

Proximate Analysis

Proximate analysis was carried out according to the procedure of Association of Official Analytical Chemist (A.O.A.C., 1990) for moisture, ash, crude fibre and crude protein content. The carbohydrate was calculated by difference method (A.O.A.C., 1990) by subtracting the sum (g/100g dry matter) of crude protein, crude fat, ash and fibre from 100g. The caloric value was determined based on the Atwater factor (FAO, 2003).

Statistical analysis

Mean ± standard deviation and spearman correlation of aflatoxin concentration against nutrient composition were calculated using SPSS software. The statistical level of significance was fixed at $P < 0.05$ (95%).

RESULTS

Impact of Fungal Contamination and Aflatoxin B_1 Concentration on the Nutrient Composition of Cereals from Agroecological Zones in Nigeria

Table 1 provides a comprehensive overview of fungal contamination, aflatoxin levels, nutrient composition, and their correlations in cereals (sorghum, maize, and rice) from different savannah zones in Nigeria. The data reveals notable differences in contamination levels across regions and crop types.

The fungal contamination, expressed in colony forming units per gram (CFU/g), varied significantly across the samples. Sudan Savannah sorghum market samples exhibited the highest fungal load (1.26×10^5 CFU/g), indicating significant fungal. Conversely, South Guinea Savannah rice samples had the lowest fungal contamination (8.33×10^2 CFU/g).

Aflatoxin levels also showed substantial variability. Derived Savannah sorghum farm samples recorded the highest aflatoxin concentration (81.47 ± 5.14 µg/kg). Sudan Savannah sorghum farm samples also had elevated aflatoxin levels (35.4 ± 11.44 µg/kg), and South Guinea Savannah maize samples, on the other hand, displayed relatively lower aflatoxin levels (17.60 ± 27.09 µg/kg).

Moisture content displayed a positive correlation with aflatoxin levels in several samples, such as sorghum from the Derived Savannah ($r = 0.998$, $p < 0.05$). Ash content, representing the mineral content of the cereals, showed varying correlations with aflatoxin levels. In sorghum samples from the Derived Savannah, ash content was negatively correlated with aflatoxin levels ($r = -0.614$). The fat content of cereals showed inconsistent correlations with aflatoxin levels. For example, in sorghum from the Derived Savannah, fat content had a weak negative correlation with aflatoxin levels ($r = -0.371$), suggesting minimal impact. However, in other cases, such as sorghum market samples from the Sudan Savannah, the correlation was stronger ($r = -0.334$). Fiber content exhibited mixed correlations with aflatoxin levels. In sorghum from the Derived Savannah, fiber content showed a negative correlation ($r = -0.965$), indicating a reduction in fiber content in heavily contaminated samples. This could result from fungal enzymes degrading cellulose and other structural components during contamination. Such degradation not only impacts the nutritional value but could also alter the textural quality of the cereals. Protein content showed both positive and negative correlations with aflatoxin levels depending on the sample. For instance, in sorghum from the Derived Savannah, a strong negative correlation ($r = -0.785$) was observed, suggesting that fungal contamination leads to protein degradation. Carbohydrate content consistently displayed strong negative correlations with aflatoxin levels, particularly in sorghum from the Sudan Savannah ($r = -0.971$).

The correlations between aflatoxin levels and nutrient composition underscore the destructive impact of fungal contamination on cereal quality. Fungal metabolism depletes essential nutrients like carbohydrates, proteins, and minerals, reducing the nutritional and economic value of the crops. Additionally, increased moisture content exacerbates contamination risks, highlighting the importance of proper post-harvest practices.

Table 1 Colony Forming Unit per gram, Aflatoxin B_1 level, nutrient composition and Pearson correlation coefficient in Sorghum of Sudan Savannah (Ajingi, Kano), Rice and Sorghum of South Guinea Savannah (Baruten, Kwara and Mokwa, Niger respectively), and Derived Savannah (Guma, Benue).

Crop	Savannah Zone	State (LGA)	Mean CFU/g	Aflatoxin Levels (µg/kg)	Mean ± SEM Nutrient Composition (Pearson Correlation Coefficient)					
					Moisture	Ash	Fat	Fiber	Protein	Carbohydrate
SF	SdS	Kano (Ajingi)	1.76×10^4	35.4 ± 11.44	18.28 ± 0.2 (.758)	2.02 ± 0.27 (.854)	2.38 ± 0.13 (.537)	3.36 ± 0.12 (.999)	9.32 ± 0.12 (.959)	64 ± 0.51 (-.971)
SM	SdS	Kano (Ajingi)	1.26×10^5	16.03 ± 18.12	15.54 ± 0.25 (-.561)	2.98 ± 0.41 (.404)	2.78 ± 0.29 (.934)	3.57 ± 0.13 (-.982)	10.47 ± 0.21 (-.208)	64.06 ± 0.3 (.711)
RM	SGS	Kwara (Baruten)	5.70×10^3	17.60 ± 27.09	16.47 ± 2.02 (-.479)	1.16 ± 0.02 (-.706)	2.26 ± 0.05 (.597)	2.47 ± 0.11 (-.635)	9.73 ± 0.93 (-.335)	63.83 ± 0.79 (-.206)
RS	SGS	Kwara (Baruten)	8.33×10^2	37.20 ± 39.05	13.04 ± 1.09 (.979)	1.19 ± 0.03 (-.390)	2.30 ± 0.01 (.308)	2.50 ± 0.10 (.743)	9.17 ± 0.06 (.546)	61.50 ± 0.61 (-.670)
SM	SGS	Niger (Mokwa)	3.70×10^4	32.67 ± 31.50	8.00 ± 0.00 (.[a])	3.30 ± 0.00 (.[a])	2.43 ± 0.02 (-.334)	3.63 ± 0.03 (-.320)	10.81 ± 0.02 (-.649)	71.25 ± 0.01 (-.506)
SS	SGS	Niger (Mokwa)	3.51×10^4	18.87 ± 27.68	10.40 ± 0.10 (.845)	3.17 ± 0.06 (-.999*)	2.52 ± 0.02 (.931)	3.68 ± 0.01 (-.040)	10.41 ± 0.02 (.957)	69.21 ± 0.03 (-.729)
SM	DS	Benue (Guma)	3.23×10^4	19.83 ± 26.49	11.07 ± 0.06 (-.955)	2.92 ± 0.07 (-.614)	2.61 ± 0.01 (-.371)	3.40 ± 0.10 (-.785)	10.22 ± 0.02 (-.785)	69.28 ± 0.03 (-.851)
SS	DS	Benue (Guma)	3.18×10^4	19.00 ± 29.51	12.48 ± 0.02 (.898)	2.97 ± 0.12 (.998*)	2.22 ± 0.03 (-.440)	3.26 ± 0.02 (-.965)	9.87 ± 0.01 (.898)	68.45 ± 0.01 (-.998*)
SF	DS	Benue (Guma)	3.91×10^4	81.47 ± 5.14	12.54 ± 0.47 (.361)	3.10 ± 0.10 (.759)	2.24 ± 0.21 (.504)	3.27 ± 0.02 (.864)	10.32 ± 0.02 (.998*)	67.37 ± 0.04 (-.995)

Key: DS – Derived Savannah (Gashaka, Taraba), SdS – Sudan Savannah (Ajingi, Kano), SS – sorghum store, SM – sorghum market

a. Cannot be computed because at least one of the variables is constant.

*. Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

The findings of this study reveal significant fungal contamination and aflatoxin B_1 levels in maize, rice, and sorghum from Guinea, Sudan, and Derived Savannah zones of Nigeria, with notable implications for food safety and nutrient composition. Aflatoxin B_1 levels exceeded the National Agency for Food and Drug Administration and Control (NAFDAC) regulatory limit of 20 µg/kg for cereals in several samples, particularly in sorghum from the Derived Savannah. Such high levels of aflatoxin pose serious health risks, including hepatotoxicity and carcinogenicity, especially in regions reliant on these staples for dietary needs (NAFDAC, 2021).

Sorghum from the Derived Savannah exhibited the highest aflatoxin B_1 levels and corresponding nutrient depletion. Carbohydrate content showed a strong negative correlation with aflatoxin B_1 concentrations, indicating that fungal metabolism depletes available carbohydrates. Protein content was also significantly reduced reflecting proteolytic activity by aflatoxin-producing fungi such as *Aspergillus flavus*. These nutrient losses not only compromise the nutritional value of sorghum but also exacerbate food insecurity in affected populations, as sorghum is a major source of energy and protein.

Maize samples from the Sudan Savannah had the highest fungal contamination, although, aflatoxin B_1 levels remained below the NAFDAC threshold. This highlights the potential for contamination to escalate under poor storage conditions, as moisture content was positively correlated with aflatoxin B_1. Effective storage interventions, such as reducing moisture and improving aeration, are critical to mitigating the risk of aflatoxin accumulation in maize.

Rice from the Guinea Savannah exhibited relatively low fungal contamination but recorded high aflatoxin B_1 concentrations, exceeding NAFDAC standards. This suggests that even minimal fungal growth can result in substantial aflatoxin synthesis, potentially due to favorable environmental factors like humidity during storage. Correlation analysis indicated a lesser impact on carbohydrate and protein content in rice compared to sorghum and maize, but the elevated toxin levels pose severe risks to consumer health.

These findings are consistent with previous studies, such as Makun *et al*. (2009), which emphasize the susceptibility of cereals to aflatoxin contamination under suboptimal storage conditions. Regulatory bodies like NAFDAC and the European Commission recommend stringent post-harvest handling and storage protocols to limit contamination (EC Regulation No. 1881/2006; NAFDAC, 2021). Strategies such as proper drying, hermetic storage, and the use of aflatoxin-reducing agents are essential to minimize contamination risks and ensure compliance with safety standards.

CONCLUSION

Overall, this study underscores the urgent need for targeted interventions to mitigate fungal contamination and aflatoxin accumulation in cereals, particularly in regions with high environmental susceptibility. Strengthening regulatory enforcement, raising awareness among farmers and traders, and investing in storage infrastructure are vital steps toward preserving food safety and nutritional quality in Nigeria's cereal crops.

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ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF ISOLATED BIOACTIVE PEPTIDES FROM FERMENTED AFRICAN OIL BEANS

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ABSTRACT

Fermented African condiments are traditional seasoning and flavor enhancers used across various African cultures. Formulation of these condiments usually involves fermentation of indigenous legumes which are then used to enhance flavor, improve preservation, and boost nutritional value by adding beneficial probiotics. The fermentation process releases bioactive peptides—short chains of amino acids that can modulate various biological processes. These peptides help alleviate oxidative stress by neutralizing free radicals and reduce inflammation by inhibiting pro-inflammatory pathways, making them beneficial for managing chronic conditions. This study focuses on the antioxidant and anti-inflammatory activities of isolated bioactive peptides from fermented African oil beans. Bioactive peptides were isolated through column chromatography and several fractions were collected. Protein content was estimated by the Bradford method, followed by *in vitro* and *in vivo* assays to assess the antioxidant and anti-inflammatory properties. The ferric reducing antioxidant power (FRAP) and 2, 2 diphenyl-1-picrylhydrazyl radical scavenging assays revealed strong antioxidant activity. Anti-inflammatory activity was also significant, with some fractions inhibiting protein denaturation by 75.22%, 57.24% and 61.54% comparable to diclofenac (84.76%). *In vivo* tests using the albumin -induced paw edema model in rats further supported the anti-inflammatory potential of these fractions. Sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) analysis was conducted and revealed the presence of low-molecular-weight peptides which are likely responsible for the observed bioactivities. These findings indicate that fermented African bean seeds-derived bioactive peptides possess significant therapeutic potential for reducing oxidative stress and inflammation.

Keywords; bioactive peptides, African oil beans, antioxidant, anti-inflammatory potential.

INTRODUCTION

Consumers have become well aware of the relationship between diet and health. The knowledge of the role of diet beyond basic nutrition has gained increased attention. Food which not only is intended to satisfy hunger and provide essential nutrients, also contributes to the prevention of nutrition-associated diseases, reduces health risks, and improve human well-being (Betoret *et al*., 2011; Gupta and Abu-Ghannam 2011; Harnedy and FitzGerald 2011).

The metabolism of Food in the body releases specific molecular functional groups or their derivatives. These specific groups are involved in the therapeutic activities of functional foods and nutraceuticals (Agyei and Danquah 2012).

Ugba is a traditionally fermented food made from the seeds of the African oil bean tree (*Pentaclethra macrophylla*), commonly found in West Africa, particularly in Nigeria (Achinewhu, 1986). It is highly regarded among the Igbo people as a staple in many traditional dishes and ceremonies (Ogueke *et al.,* 2010). The production of fermented oil bean tree (ugba) involves several steps including boiling, de-hulling and fermenting (Enujiugha *et al.,* 1998). Fermented oil bean seeds are a good source of protein, edible oils and fats.

Proteins, besides being essential for growth, also contain peptides that offer various health benefits (Korhonen and Pihlanto, 2006). These bioactive peptides, consisting of 2–30 amino acids linked by peptide bonds, are typically embedded within proteins and must be released through digestion or microbial fermentation (Jakubczyk *et al*., 2020). They are often rich in amino acids such as proline, arginine, and lysine, as well as hydrophobic residues and once released, exhibit various biological activities, including antioxidant, antimicrobial, antihypertensive, and immunomodulatory effects (Wang *et al*., 2019).

In therapeutic applications, bioactive peptides have gained attention in pharmaceuticals and functional food production due to their ability to act like hormones or drugs. Peptides are now preferred over traditional drugs for their specific biological activities, lower toxicity, and reduced side effects. Considered to show more specialized activity towards the target tissue at low concentrations, they have little or no toxicity effects. (Akbarian *et al.,* 2022).

MATERIALS AND METHODS

Sample collection

100g of fermented oil bean seed was purchased from a store in Kure market, Bosso Local Government, Niger state.

Preparation of sample

The freshly fermented African oil bean seed was soaked in a plastic tube overnight with distilled water under room temperature, then later removed from the tube and homogenized into a smooth fluid then filtered into a conical flask.

Experimental animal

Wistar albino rats, weighing between 150 and 500 grams, were purchased from the Animal House Center of the Department of Biochemistry, Federal University of Technology, Minna. The rats were given a week to acclimatize in the animal house of the Federal University of Technology, Minna.

Extraction of sample

Column was set on a retort stand, then the buffer was mixed with the silica gel in a conical flask then hydrated with distilled water which was pour on the column(mobile phase). 20ml of oil bean seed extract was mixed with silica gel to adsorb the sample to increase the surface area so that separation can be effective (stationary phase). The samples were collected in different filtrates little by little (size by size) until there was a colorless filtrate. Then a spectrophotometer was used to check the absorbance of each test tube to know which of them have same numbers of peaks, samples with same peaks were pooled together into one tube meaning they have same components. Same steps were taken until all the tubes with different components were collected.

Determination of antioxidant activity

The antioxidant activity of the oil been seed extracts was estimated using the DPPH radical scavenging assay and ferric reducing antioxidant power (FRAP) methods as described by Oyaizu (1986).

Determination of anti-inflammatory activity

Estimation of anti-inflammatory activity using protein denaturation assay was done according to the method described by Gambhire *et al.,* 2009 with some modifications as described in Gunathilake *et al.* (2018).

Determination of anti-inflammatory activity using Egg albumin-induced paw edema in rats

The anti-inflammatory activities of the extracts were evaluated according to the method described by Winter *et al*., (1963).

Determination of Protein Concentration

Protein Concentration was determined according to the method described by Bradford (1979).

Determination of molecular weight

The proteomic analysis followed the procedure of Weber and Osborn (1969), with an update by Aslam *et al.* (2016), using SDS-PAGE.

Statistical Analysis

The data obtain were analyzed using IBM Statistical Package for Social Science (SPSS) version 23 at the Degree of Freedom, $P < 0.05$. Statistical differences between means was compared using paired Duncan HSD. Differences in means were considered statistically significant at $p < 0.05$.

RESULTS

Determination of antioxidant activity

Among samples tested for in vitro antioxidant Ferric Reducing Antioxidant Power (FRAP) activity ascorbic had the highest value with 95.36±0.81, F4 had the second highest value with 71.44±0.30 while sample F2 had the least value of 25.35±0.21 at 1000µg/ml concentration. At concentrations of 500µg/ml, sample F4 had the second highest level of activity of 60.09±0.77 while sample F2 had the least value of 18.17±0.26 with ascorbic acid maintaining higher levels of *in vitro* antioxidant activity with value of 89.09±0.77. The same trend is still observed at 250µg/ml as sample F4 had the second highest value of 52.07±0.36 while sample F2 still maintained the least value at 6.93±0.39 with ascorbic acid maintaining a higher level with values of 84.76±0.66. At concentrations of 125µg/ml and 62.5µg/ml, ascorbic acid had significantly higher levels of in vitro antioxidant FRAP activity measuring as high as 80.64±0.64 and 64.36±0.21 respectively sample F4 had values of 17.51±0.39 and 4.81±0.60 while there was no noticeable antioxidant activity in sample F2.

Table 1: Ferric Reducing Antioxidant Power (FRAP) of bioactive peptides from fermented African oil bean seeds.

Sample	1000µg/mL	500µg/mL	250µg/mL	125µg/mL	62.5µg/mL
F1	31.93±0.18^{c}	24.86±0.27^{c}	13.48±0.28^{c}	4.69±0.64^{b}	**0±0.00^{a}**
F2	25.35±0.21^{a}	18.17±0.26^{a}	6.93±0.39^{a}	0±0.00^{a}	**0±0.00^{a}**
F3	46.69±0.64^{b}	38.73±0.68^{e}	26.94±0.39^{e}	17.51±0.39^{d}	**4.81±0.60^{c}**
F4	71.44±0.30^{e}	60.09±0.77^{f}	52.07±0.36^{f}	34.78±0.27^{e}	**22.97±0.67^{d}**
F5	29.93±0.61^{c}	23.38±0.19^{b}	14.77±0.45^{c}	7.94±0.62^{c}	**1.67±0.11^{b}**
F6	32.13±0.61^{c}	25.81±0.41^{c}	19.01±0.43^{d}	12.99±0.21^{d}	**3.54±0.23^{c}**
F7	37.81±0.60^{d}	30.98±0.66^{d}	21.78±0.44^{d}	15.68±0.64^{d}	**5.1±0.54^{c}**
F8	34.62±0.60^{c}	27.64±0.21^{c}	16.42±0.37^{c}	9.05±0.51^{c}	**2.10±0.12^{b}**
Ascorbic acid	95.36±0.81^{g}	89.09±0.77^{g}	84.76±0.66^{g}	80.64±0.64^{g}	**64.36±0.21^{g}**

Determination of anti-oxidant DPPH activity using *in vitro* assay

In vitro antioxidant DPPH activity shows a general downward trend as represented by the line graph. It can clearly be seen that with decrease in concentration there is also decrease in DPPH antioxidant activity and highest levels of DPPH antioxidant activity noticed in ascorbic acid. However, sample F4 had the highest DPPH activity among the samples which was significantly higher than those of the other groups.

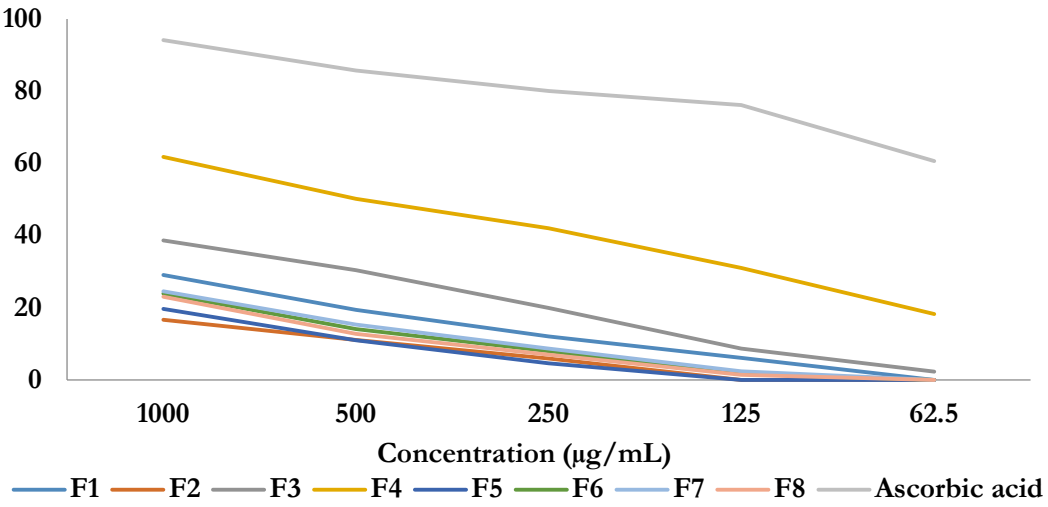


Figure 1: DPPH activity of bioactive peptides from fermented African oil bean seed

Determination of anti-inflammatory activity using Protein denaturation

The graph in Figure 2 shows the protein denaturation power of different samples at different concentrations. It shows that with decrease in concentrations there is also significant decrease in protein denaturation of these samples. However, diclofenac maintained a higher level of protein denaturation as compared against other samples, it shows a marked decrease in protein denaturation from the 500µg/ml concentration point as there is a sharp decrease in activity as shown by the graph. Among the other samples, F4 maintained a higher level of protein denaturation with F1 having the least protein denaturation at 1000µg/ml. At 62.5µg/mL F1, F2, F5, F6 and F8 showed no protein denaturation activity as against F4 which still had a significantly high level of protein denaturation.

Determination of Protein content using Bradford assay

The chart in Figure 3 represents the protein concentration in each sample. From the chart it was observed that among the various samples, F3 had the highest protein concentration when compared against other samples with F6 also having a high level of protein concentration with F5 having the least concentration of protein among samples.

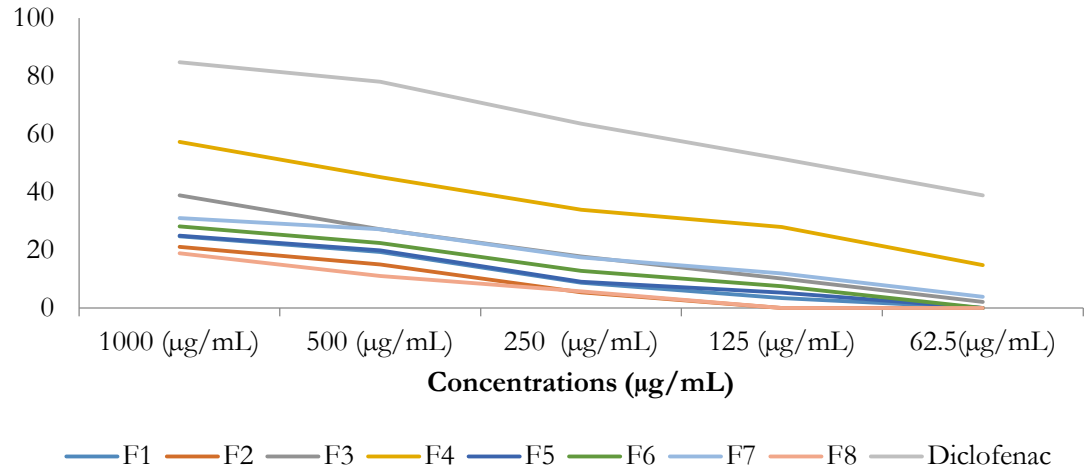


Figure 2: Anti-inflammatory activity of bioactive peptides from fermented African oil bean seed using Protein Denaturation

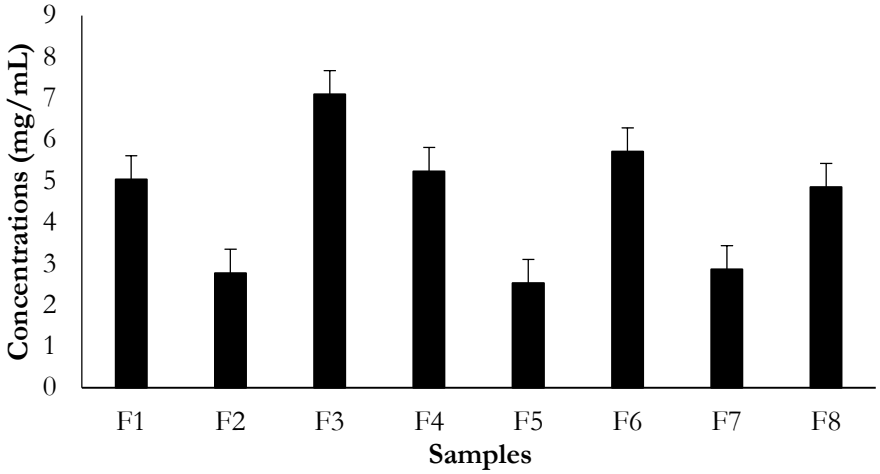


Figure 3; protein concentration of bioactive peptides from fermented African oil bean seed

Determination of Anti- Inflammatory Activity Using Egg Albumin-Induced Paw Oedema

The table below shows the *in vivo* anti-inflammatory activity of samples F1 (500µg/ml), F5 (500µg/ml), negative control and indomethacin (standard drug). The result showed that both fractions had some level of effect on the oedema nearly mimicking the standard drug especially after 2 hours. After 8 hours, F5 range exhibited the highest effect compared to F1.

Table 2: In vivo Anti- Inflammatory Activity of fermented oil bean seeds on Egg Albumin-Induced Paw Oedema

Concentration	0 hr	1 hr	2 hr	4 hr	6 hr	8 hr
500 mg/kg F1	0.30	0.70	0.62	0.54	0.52	0.44
500 mg/kg F5	0.23	0.66	0.53	0.50	0.46	0.40
Control	0.29	0.58	0.76	0.84	0.81	0.77
Indomethacin	0.27	0.66	0.57	0.54	0.48	0.49

Molecular weight of Bioactive Peptide from oil bean seeds

Lane 1 contains the molecular weight markers from the Bio-Rad Dual Xtra standard, which range from 250 KDa down to 2 KDa. These markers serve as a reference for the determination of molecular weights in the unknown protein samples. Each marker is associated with a specific Relative Front (Rf), or migration distance, which is critical for estimating molecular
weights of the unknown proteins in other lanes.

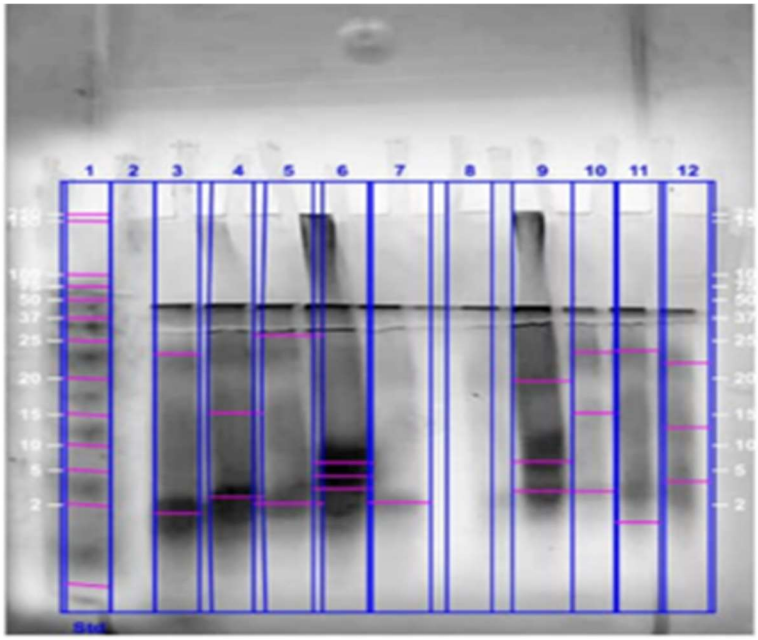
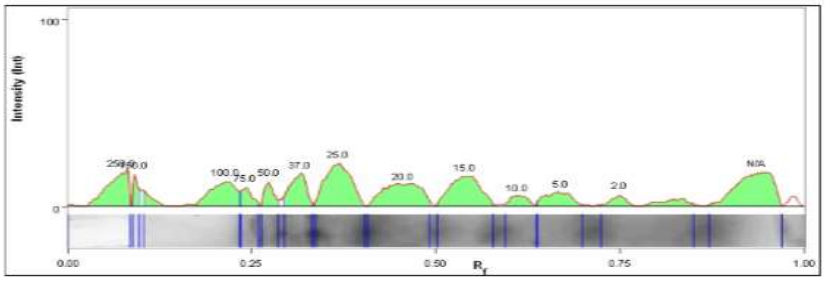


Figure 5: SDS-PAGE on Molecular Weight of Bioactive Peptide of oil beans extracts

Lane 1 - Bio-Rad Dual Xtra

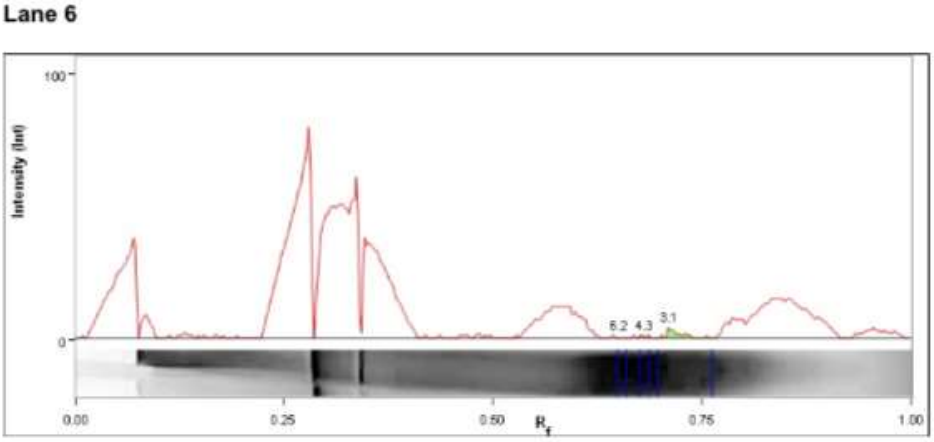


Band No.	Band Label	Mol. Wt. (KDa)	Relative Front	Adj. Volume (Int)	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1		250.0	0.075	10,692	338,832	N/A	N/A	9.5	9.3
2		150.0	0.092	2,268	40,104	N/A	N/A	2.0	2.0
3		100.0	0.216	11,376	489,996	N/A	N/A	10.1	9.9
4		75.0	0.243	2,808	82,440	N/A	N/A	2.5	2.4
5		50.0	0.275	3,240	78,876	N/A	N/A	2.9	2.8
6		37.0	0.317	7,632	121,536	N/A	N/A	6.8	6.6
7		25.0	0.369	16,272	208,728	N/A	N/A	14.5	14.1
8		20.0	0.457	12,960	262,368	N/A	N/A	11.5	11.3
9		15.0	0.541	13,536	248,004	N/A	N/A	12.0	11.8
10		10.0	0.612	2,700	140,832	N/A	N/A	2.4	2.3
11		5.0	0.671	5,472	200,520	N/A	N/A	4.9	4.8
12		2.0	0.751	5,040	401,868	N/A	N/A	4.5	4.4
13		N/A	0.937	18,432	337,680	N/A	N/A	16.4	16.0

Lane Background	Lane background subtracted with disk size: 10
Lane Width	3.05 mm
Regression Equation	A single equation is not available for this method

Figure 6: Lane and Band Analysis (Lane 1)

Lane 6 containing Bioactive peptides from African oil bean seed contains three protein band, with the first having Molecular weight of 6.2 KDa, and a Relative Front (Rf) value of 0.652, the second has molecular weight of 4.3Kda with Rf value of 0.683 and the last one which have a molecular weight of 3.1KDa and Rf value of 0.713. Band 3 occupies 0.6% of the lane, while the other contributes negligibly.



Band No.	Band Label	Mol. Wt. (KDa)	Relative Front	Adj. Volume (Int)	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1		6.2	0.652	0	37,224	N/A	N/A	0.0	0.0
2		4.3	0.683	188	37,318	N/A	N/A	11.8	0.1
3		3.1	0.713	1,410	217,892	N/A	N/A	88.2	0.6

Lane Background	Lane background subtracted with disk size: 10
Lane Width	3.98 mm
Regression Equation	A single equation is not available for this method

Figure 7: Lane and Band Analysis (Lane 6)

DISCUSSION

This study investigated the antioxidant and anti-inflammatory activities of bioactive peptides present in fermented African oil bean seed (ugba). The results demonstrate significant antioxidant and anti-inflammatory potential, which aligns with and expands upon recent findings in the field of functional foods and bioactive peptides. The ferric reducing antioxidant power (FRAP) assay results reveal that sample F4 exhibited the highest antioxidant activity among the tested extracted fermented African oil bean (ugba) samples, with a value of 71.44±0.30 at 1000 µg/ml concentration. This finding is particularly noteworthy as it suggests that certain bioactive peptides in fermented African oil bean (ugba) possess strong electron-donating capabilities, which is a crucial mechanism in combating oxidative stress. The potent antioxidant activity of F4 aligns with recent studies on other fermented legumes. For instance, Zhang *et al.,* (2021) reported enhanced antioxidant properties in fermented soybean products due to the release of bioactive peptides during fermentation.

The DPPH radical scavenging assay further corroborated the antioxidant potential of these samples, with F4 consistently demonstrating superior activity across various concentrations. This observation is consistent with the findings of Adebo *et al.,* (2020), who noted that fermentation significantly improves the antioxidant capacity of legumes through the production of bioactive compounds.

The protein denaturation inhibition assay results indicate that sample F4 exhibited the highest anti-inflammatory activity among the extracted African oil bean (ugba) samples tested. This finding suggests that certain bioactive peptides in fermented African oil bean (ugba) may possess the ability to stabilize protein structures, thereby potentially reducing inflammation. The observed anti-inflammatory effect, although lower than that of diclofenac, is significant considering the natural origin of the peptides.

These results are in line with recent studies on bioactive peptides derived from other fermented legumes. For example, Liu *et al.,* (2023) reported that peptides from fermented black soybeans demonstrated notable anti-inflammatory effects

in vitro and in vivo. Similarly, Ogundele *et al.,* (2022) found that fermented cowpea peptides exhibited significant anti-inflammatory properties, supporting the potential of fermented legumes as natural anti-inflammatory agents.

The protein concentration analysis revealed that sample F3 had the highest protein content, followed closely by F6, while F5 had the lowest. This variability in protein content among different extracted African oil bean (ugba) samples is consistent with findings by Okorie & Olasupo, (2023), who reported that protein content in fermented African oil bean seeds can vary significantly depending on fermentation time and microbial composition. The high protein content observed in some samples could be indicative of extensive protein hydrolysis during fermentation, leading to the release of bioactive peptides. This hypothesis is supported by research from Nwosu & Ojimelukwe (2021), who found a positive correlation between protein hydrolysis and the generation of bioactive peptides with antioxidant properties in fermented legumes.

The in vivo anti-inflammatory activity results showed that both F1 and F5 samples (500µg/mL) exhibited higher anti-inflammatory activity compared to the control in the first hour. However, the effect diminished over time, with the control showing higher activity after the second hour.

This initial anti-inflammatory effect aligns with recent findings by Emeka *et al.,* (2023), who reported rapid but short-lived anti-inflammatory effects of bioactive peptides derived from fermented plant sources. They suggested that this could be due to the quick absorption and metabolism of these peptides in the body. The observed decrease in anti-inflammatory activity over time in our study is intriguing and warrants further investigation. It could potentially be explained by the findings of Eze *et al.,* (2024), who proposed that some bioactive peptides might have a biphasic effect, initially suppressing inflammation but potentially promoting it over longer periods, depending on the physiological context. The strong antioxidant activity, particularly of sample F4, suggests that specific fermentation conditions or bacterial strains might be optimal for enhancing the functional properties of African oil bean seeds. The complex interplay between in vitro and in vivo antioxidant activities, as well as the time-dependent nature of the anti-inflammatory effects, highlights the need for further research.

The molecular analysis of fermented oil beans seed extracts reviewed that Band 3 with 3.1 KDa could have a substantial amount of bioactive peptides, while Bands 1 and 2 showed relatively small quantities, possibly indicating trace amounts of peptides at 6.2 Kda and 4.3 KDa. The antioxidant and anti-inflammatory activity displayed in the sample maybe due to peptides from band 3 which was suggested to have more of the bioactive peptides, according to Udenigwe *et al.,* (2012) that suggested that low molecular weight peptides are more likely to be bioavailable and bioactive compared to larger peptides.

CONCLUSION

From this study, aqueous extracts of fermented oil bean seeds showed promising antioxidant potentials, which may be as a result of the bioactive peptides present in the extract.

The anti-inflammatory properties of the peptides evaluated using the inhibition of protein denaturation demonstrated a potential to inhibit protein denaturation and inflammatory mediators.

This study has provided valuable insights into the antioxidant and anti-inflammatory properties of bioactive peptides present in fermented African oil bean seed (ugba).

Further confirming the findings of this research was the results from SDS PAGE suggesting that present in the samples were three peptides with band 3.2kva having more of the bioactive peptides. Future studies is however required to isolate of specific peptide sequences responsible for the observed bioactivity.

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