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Bacteriological Quality of Locally Fermented *Pentaclethra Macrophylla* Sold in Ikwo Ebonyi State

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Abstract:

This investigation was carried out to determine the bacteriological quality of locally processed ugba sold in Ikwo market in Ebonyi State. A total of four different samples of ready to eat African oil bean were purchased from different vendors in the market. Using standard microbiological procedures, 10g each of the samples were homogenized in 90 ml of sterile distilled water, serially diluted, plated and incubated at 37^{o}C for 24 hours. The total viable counts were estimated using the colony counter while Kirby Bauer disk diffusion method was used for the determination of the antimicrobial susceptibility. The organisms found were *Salmonella sp*, *Klebsiella sp*, *E.coli* and *Bacillus sp*. The total bacterial count of *Pentaclethra macrophylla* samples A, B,C and D were 1.8 x 10^4, 5.6 x 10^4, 6.2 x 10^4 and 7.2 x 10^4 cfu/g. Antimicrobial susceptibility tests conducted on the isolates showed that they were susceptible to imipenem and ciprofloxacin but resistant to cefotaxime and tazobactam/piperacillin antibiotics.

Keywords: Ciprofloxacin, bacteriological, susceptibility, fermentation.

Introduction

Pentaclethra macrophylla also known as African oil bean tree steadily produces seeds that are sliced and fermented to produce a popular Nigerian delicacy 'African salad'. It is known as Ugba by the Igbos and other ethnic groups in the southeastern part of Nigeria (Njoku *et al*., 2020). Ugba is rich in proteins, vitamins, probiotics and it is produced by alkaline fermentation of the oil bean seeds. It is often eaten as a snack or as food condiment. It is served as an important food item in various traditional events in Igbo land (Anioke *et al*., 2023). Fermentation is applied widely in food production, industrial microbiology and biotechnological processes. Although fermentation can be natural, they can be controlled and the microorganisms play significant roles in changing the physical, organic and sensory properties of the substrates (Onwuakor *et al*.,2024). During this process, microorganisms can generate favourable and undesirable chemical changes in the fermentation end products (Omeh *et al*.,2023). However, controlled fermentation enhances microbial and nutritional quality. Like when starter culture fermentation resulted in improved ash content and protein when compared to the traditional methods (Agbo *et al*.,2024).Fermentation significantly enhances amino acid composition. In a recent study, total amino acid increased from around 82.7g/100g protein in raw seeds to approximately 109.2g/100g protein after 120 hours (or 5 days) of fermentation which is an increment by 32% (Ogbuagu *et al*., 2020). Like most other fermented food products, the occurrence and growth of pathogens in ugba cannot be ruled out as general hygienic conditions of the processors, water used, the equipment and other raw materials cannot be guaranteed to be free of potential pathogens. Achieving a safe level of pathogens during production and distribution using Hazard Analysis Critical Control Points (HACCP) program will be a good strategy. The aim of this work was to investigate the microbial load of ugba while using antimicrobial susceptibility test to determine the

antibiotics that will be relevant in the treatment and control of resistant strains.

Materials and methods

Sample collection

Four (4) different fermented samples of *Pentaclethra macrophylla* were purchased from different vendors in the different markets in Ikwo, making a total of 12 samples altogether. Clean and sterile polythene bags were used to transport the samples to the Microbiology Laboratory of Alex Ekwueme Federal University Ndufu-Alike Ikwo for subsequent analysis

Media preparation.

Media used such as Salmonella-shigella agar, mannitol salt agar, Mueller Hinton agar, eosin methylene blue agar, nutrient broth and nutrient agar were all prepared according to manufacturer's instructions.

Antibiotics used:

Tazobactam/ piperacillin (110 µg), Imipenem(IMI10 µg), Cefotaxime(30 µg), Polymyxin B(300 µg) and ciprofloxacin (5 µg) were used in this study.

Sample Preparation

Exactly ten grams (10g) of *Pentaclethra macrophylla* was homogenized in 90 ml of sterile distilled water and used successfully for 10-fold serial dilution. Aliquot (0.1 ml) was plated out on pre-prepared culture media plates and incubated at 37°C for 24 hours.

Characterization of the Isolated Organisms

The characterization and identification of the isolates were done using standard microbiological procedures. The different colonies on the different media used were observed and noted. The morphology and arrangement of the microbial cells were recorded. The isolates were subjected to gram staining test and other biochemical tests for further identification.

Total bacterial Count

Equal volumes (1 ml) of 10^{-1} to 10^{-4} dilutions of each sample were pour-plated into a sterile petri dish containing nutrient agar. The plates were incubated at 37°C for 24 hours. The colony counter was used to count the colonies that grew and expressed in colony forming units.

Antibiotic susceptibility test

The Kirby-Bauer diffusion method was used. Sterile nutrient broth were prepared, inoculated with the test organisms, and incubated at 37°C for 24 hours. Using sterile swab sticks, 0.5 McFarland standard of the cultures were uniformly spread on pre-prepared Mueller-Hinton agar and left to stand for 15 minutes. Then the antibiotics sensitivity discs were carefully placed on the Mueller-Hinton agar plates using sterile forceps and incubated at 37°C for 24 hours. The diameter of zone of inhibition were measured with the aid of meter rule and recorded.

Results

Table 1: Colony morphology and biochemical tests of the isolates

Isolates	shape	Gram stain	Catalase	Indole	Urease	Methyl red	VP	Citrate	Sugar F	gelatin	Starch hyd	Identified organism
PMSA	Rod	–	+	+	–	+	–	–	+	–	–	*E. coli*
PMSB	Rod	+	+	–	–	–	+	+	+	+	+	*Bacillus* sp
PMSC	Rod	-	+	–	+	–	+	+	+	–	–	*Klebsiella* sp
PMSD	Rods	–	+	–	–	+	–	+	+	–	–	*Salmonella* sp

Gram = gram reaction, VP- Voges Proskauer test, Gelatin- gelatin liquefaction test, Sugar F- Sugar fermentation test, starch hyd- starch hydrolysis test

Table 2: Total bacterial count fond in the samples

Food samples	Number of colonies	Bacterial counts	Isolated Bacteria
PMSA	18	$1.8x10^4$	*Bacillus* sp, *Salmonella* sp
PMSB	56	$5.6x10^4$	*Bacillus* sp
PMSC	62	$6.2x10^4$	*E. coli*
PMSD	72	$7.2x10^4$	*Salmonella* sp and *Klebsiella* sp

Table 3: Results of antibiotic susceptibility testing of *Salmonella* and *Bacillus* species from *Pentaclethra macrophylla* Sample A

	Inhibition zone Diameter (mm)				
Isolates	TZP(110µg)	PB (300µg)	CIP (5µg)	CTX (30µg)	IMI (10µg)
Salmonella sp	–	–	30	-	20
Bacillus sp	–	18	29	-	31

Key: CTX- Cefotaxime, PB-Polymyxin,, CIP- Ciprofloxacin, TZP-Tazobactam/Piperacillin, IMI-Imipenem,, - No inhibition

Table 4: Results of antibiotic susceptibility testing on *Bacillus* sp isolated from *Pentaclethra macrophylla* Sample B

	Inhibition zone Diameter (mm)				
Isolates	TZP(110µg)	PB (300µg)	CIP (5µg)	CTX (30µg)	IMI (10µg)
Bacillus sp	–	17	28	-	30

Key: CTX- Cefotaxime, PB-Polymyxin,, CIP- Ciprofloxacin, TZP-Tazobactam/Piperacillin, IMI-Imipenem,, - No inhibition

Table 5: Results of antibiotic susceptibility testing on *E. coli* isolated from *Pentaclethra macrophylla* Sample C

	Inhibition zone Diameter (mm)				
Isolates	TZP(110µg)	PB (300µg)	CIP (5µg)	CTX (30µg)	IMI (10µg)
E. coli	–	17	30	-	27

Key: CTX- Cefotaxime, PB-Polymyxin,, CIP- Ciprofloxacin, TZP-Tazobactam/Piperacillin, IMI-Imipenem,, - No inhibition

Table 6: Results of antibiotic susceptibility testing of *Salmonella* and *Klebsiella* species from *Pentaclethra macrophylla* Sample D

	Inhibition zone Diameter (mm)				
Isolates	TZP(110µg)	PB (300µg)	CIP (5µg)	CTX (30µg)	IMI (10µg)
Salmonella sp	–	–	29	-	19
Bacillus sp	–	15	26	-	28

Key: CTX- Cefotaxime, PB-Polymyxin,, CIP- Ciprofloxacin, TZP-Tazobactam/Piperacillin, IMI-Imipenem,, - No inhibition

Discussion

Pentaclethra macrophylla sample A showed a 100% occurrence of *Bacillus* sp and Salmonella sp, whereas *Pentaclethra macrophylla* sample B and C showed the presence of Bacillus sp and *E.coli* respectively. That of *Pentaclethra macrophylla* sample D showed the presence of *Salmonella* sp and *Klebsiella* sp. The isolation of diverse groups of microorganisms is an indicator of poor hygienic practices in the course of processing. The presence of *Salmonella* sp, *E. coli*, and *Klebsiella* sp suggests fecal contamination from the surroundings. This corroborates the work of (Ike and Emeka-Ike,2016;Udo and Ojimelukwe, 2024) who isolated *E. coli* and *Salmonella* sp from Ugba samples. It equally corroborates the work of Okorie *et al.*, 2017 who isolated *Salmonella enterica* and *Proteus mirabilis*.The presence of these organisms poses a public health risk, as these organisms are implicated in the outbreak of foodborne diseases. The total bacterial count of *Pentaclethra macrophylla* A, B, C and D were 1.8×10^4, 5.6×10^4, 6.2×10^4 and 7.2×10^4 cfu/g. The result showed that *Pentaclethra macrophylla* sample D from a different vendor had the highest microbial load while *Pentaclethra macrophylla* sample A had the lowest microbial count. The presence of different foodborne pathogens, such as *E. coli* and *S. aureus* has been reported by Anyanwu *et al.*,2016;James *et al.*,2025) who isolated *Staphylococcus* sp, *E.coli*, *Klebsiella* sp, *Bacillus* sp, *Lactobacillus* sp and other fungal species. The presence of these bacterial species in the *Pentaclethra macrophylla* samples has been noted to be risky, since the ugba is not heated again before consumption. However, its addition to soup as condiment involves heating which could eliminate these pathogens. The presence of these pathogens however, inhibits the total fermentation of the *Pentaclethra macrophylla* by the probiotic organisms. This corroborates the work of Uzoh *et al.*, 2024. However, the microbial load found in a particular food depends on different factors like the level of sanitation of producers, the environment of production, the method of production and the way the vendors handle them. *Pentaclethra macrophylla* sample Z from a different market had a higher microbial count than *Pentaclethra macrophylla* sample C. This marked difference is attributed to the fact that the former was a primary seller whereas the latter was a secondary or tertiary seller, who had been involved in several openings and repackaging of the vended ugba which is subject to chance inoculation by the microorganisms. However, the presence of these organisms in ugba could be from the raw materials especially water, used in the production process. Since fermentation of African oil bean seeds has been shown to be an alkaline process (about pH 8.2), the antimicrobial effect associated with most fermented foods because of their low pH is absent in ugba.

This corroborates the work of (Ogueke *et al.*, 2015; Olasupo *et al.*, 2016). Among the antibiotics used, it was ciprofloxacin and imipenem that showed most effective activity against *Salmonella* sp, *Bacillus* sp, *E. coli* and *Klebsiella* sp while it was resistant to cefotaxime and tazobactam/piperacillin. This was in agreement with the work of James *et al.*, 2025. It was observed from this study that the foods that are not properly handled may harbor extended-spectrum beta-lactamases. Resistance to TZP indicates possible plasmid-mediated mechanisms such as CTX-M, SHM or TEM enzymes which are mostly predominant in *Enterobacteriaceae* within West Africa (Irek *et al.*,2023). Being resistant to cefotaxime is a major concern since cephalosporins are administered to both humans and domestic animals in Nigeria, which contributes to the spread of resistant pathogens.The results showed that ciprofloxacin effectively combats these organisms as all the isolates were susceptible. The efficacy of these antibiotics suggests that the isolates may not have acquired any resistance. The isolates were susceptible to imipenem, which emphasizes on the use of carbapenems as a reliable drug against multidrug-resistant *Enterobacteriaceae*.

Conclusion

The isolates identified from the *Pentaclethra macrophylla* samples were *Bacillus* sp, *Salmonella* sp, *E. coli* and *Klebsiella* sp which showed that *Pentaclethra macrophylla* prepared under unhygienic conditions may cause gastroenteritis or food poisoning. The presence of these isolates in significant numbers poses a public health risk. Adherence to safe hygienic practices such as processing it in hygienic environments such as

sterile materials and clean water, which will extend the shelf life and improve the microbiological quality. However, *Pentaclethra macrophylla* processors and vendors should be educated. A better approach to this could be educating of the older women, who are primarily the producers and processors or training the younger ones on techniques in the processing and the need to adhere to hygienic and good manufacturing practice. Molecular characterization should be performed on their isolates to further identify them and determine their pathogenicity and virulence factors.

References

Agbo, N. J. (2024). Comparative nutritional and mineral analysis of fermented *Pentaclethra macrophylla*(ukpaka), fermented Ricinus communis(ogiri), and fermented Prosopis africana seed (okpeye). *FUDMA Journal of Sciences* 8(3):344–347.

Anioke, I.C. (2023). Nutrient changes during the fermentation of African oil bean (*Pentaclethra macrophylla* Benth) seeds. *Pakistan Journal of Nutrition* 2(5): 320 - 323.

Anyanwu, N.C.J., Okonkwo, O.L., Iheanacho, C.N. and Ajide, B., (2016). Microbiological and Nutritional Qualities of Fermented Ugba (Pentaclethra macrophylla Benth.) sold in Mbaise, Imo State, Nigeria. *Annual Research & Review in Biology* 9: 1-8

Irek, E. O., Amadi, E. S., & Ugwu, M. C. (2020). Prevalence and molecular characterization of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in Nigeria: A systematic review. *Journal of Global Antimicrobial Resistance* 22: 124–133.

James, I. C., Ofoegbu, V. O., Augustine, P. C., Ukaegbu, G. N., Oje, N. C., Chukwudiebere, I. K., Edeji, F. U., and *Pentaclethra macrophylla* Lawal, A. K.(2025).The Prevalence Of Staphylococcus Species on Locally Fermented (African Oil-Bean Seed) and its Antibiotics Sensitivity *Scientia Afrcana* 24 (5):151-158.

Njoku, H.O., Ogbulie, J.N. and Nnubia, C. (2020). Microbiological study of the traditional processing of African oil bean (Pentaclethra macrophylla Bentham) for Ugba production. *Food microbiology* 7(1): 13-26.

Ogueke CC, Anosike F, Owuamanam C I.(2015). Prediction of amino nitrogen during ugba (*Pentaclethra macrophylla*) production under different fermentation variables: A response surface approach. *Nig. Food J*. 33:61–66.

Okorie, P.C., Olasupo, N.A., Anike, F.N., Elemo, G.N. and Isikhuemhen, O.S. (2017). Incidence of enteric pathogens in ugba, a traditional fermented food from African oil bean seeds (*Pentaclethra macrophylla*). *International Journal of Food Contamination* 4(1): 12-15.

Olasupo N.A, Okorie P.C, Oguntoyinbo F.A (2016). The biotechnology of ugba, a Nigerian traditional fermented food condiment. *Front Microbiol*. 7:1153–1161.

Omeh, N.Y., Omosun, G., Adiele, I.P. And Ejiofor, U.E. (2023). Major fermentative Organisms in some Nigerian Soup Condiments. *Pakistan Journal of Nutrition* 8(3):279-283.

Onwuakor C.E., Sanni, A.I and Ukeka, E. (2024). Proximate composition, biochemical and microbiological changes associated with fermenting African oil bean (Pentaclethra macrophylla Benth) seeds. American *Journal of Microbiology Research* 2(5): 138-142.

Udo, I. I and Ojimelukwe, P. C(2024). Effect of Isolated Cultures Fermentation on the Microbial Loads of Fermented African Oil Bean Seed (*Pentachletra macrophylla*) products. *African Journal of Agricultural Science and Food Research* 15(1):43-60

Uzoh C.V,Aroh K.E,Yusuf I,Nworie C.O, Igwe P. C,Ugwu B,Ugwuocha C.S, Uwanta L.I and Onuoha N.S (2024). Effects of Selected Plant Preservatives on Microbial Load and Shelf Life of Palm Wine *Asian Journal of Food Research and Nutrition* 3(1): 85-91.

