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Antimicrobial Effects and Comparative Analysis of *Xylopia aethiopica* (Uda) and *Monodora myristica* (Ehuru) as Natural Preservatives for Abacha (African Salad)

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Abstract

This study evaluated the antimicrobial efficacy of *Xylopia aethiopica* (Uda) and *Monodora myristica* (Ehuru) as natural preservatives for *Abacha* (African salad), a traditional Nigerian ready-to-eat food that is highly susceptible to microbial contamination. *Abacha* samples were obtained from two vendors, treated with different concentrations (2% and 5%) of Uda and Ehuru, and stored for 4 h at ambient temperature. Microbial analyses were conducted immediately upon arrival and after storage. The pH was evaluated, and microbial load was assessed using nutrient agar and selective media, including MacConkey agar, Mannitol Salt Agar, Eosin Methylene Blue agar, and Salmonella–Shigella agar, followed by biochemical tests for bacterial identification. Sensory evaluation was conducted using a 9-point hedonic scale. Five bacterial species were identified: *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, and *Enterobacter* spp. Ehuru exhibited the highest antimicrobial activity, with 5% Ehuru reducing microbial counts to near-baseline levels of 1.1×10^3 and 1.2×10^3 CFU/mL. Compared to untreated controls, which had 2.5×10^3 and 3.3×10^3 CFU/mL from vendors A and B, respectively. Uda also demonstrated inhibitory effects, but with lower efficacy and reduced sensory acceptability. *Abacha* with 2% Ehuru-treated samples recorded higher overall acceptability scores of 8, whereas Uda-treated samples were poorly rated, particularly in terms of taste and overall acceptability. These findings highlight the superior potential of Ehuru as a culturally relevant natural preservative for *Abacha*, combining effective microbial inhibition with high consumer acceptance. The adoption of indigenous spices in food preservation could reduce reliance on synthetic preservatives, thereby enhancing food safety and public health in Nigeria.

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Keywords: Abacha, food safety, natural preservatives, *Xylopia aethiopica*, *Monodora myristica*, antimicrobial activity.

Introduction

Street foods are an integral part of Nigerian culinary culture, providing affordable and convenient meals for diverse populations (Mazi et al., 2023). In developing countries, the World Health Organization estimates that street foods are among the major sources of microbial contamination and foodborne outbreaks, posing a significant challenge to food safety (Osuji, 2024). In southeastern Nigeria, Abacha (African

Salad), is one of the most widely consumed traditional street food delicacies prepared from processed cassava. Abacha is appreciated for its unique texture and tangy taste and is commonly garnished with palm oil, vegetables, and indigenous spices such as Uda (*Xylopia aethiopica*) and *Ehuru* (*Monodora myristica*). Its nutritional richness, cultural symbolism and convenience have made it a staple meal in urban centers like Enugu. Despite these benefits, Abacha is categorized as a high-risk food because it is prepared with minimal heat treatment. Such foods are susceptible to microbial contamination, often involving foodborne pathogens such as *Salmonella spp.*, *Escherichia coli*, and *Staphylococcus aureus* (Tampa et al., 2025). That pose significant public health concerns especially in urban centers like Enugu where *Abacha* is commonly eaten (Barrios-Garcia & Salinas-Chavira, 2024, Enabulele *et al*., 2014,). Currently, synthetic preservatives such as sodium benzoate and nitrates are widely used in the food industry to extend shelf life and prevent microbial spoilage. While effective, their long-term consumption is associated with adverse health effects, including hypersensitivity reactions and carcinogenic potential (Sulieman *et al.*, 2023). This has triggered a growing interest in natural, plant-derived preservatives that are safer, eco-friendly, and culturally acceptable (Abdullahi, 2019). Spices, in particular, offer dual benefits as flavor enhancers and antimicrobial agents, making them promising alternatives to synthetic additives.

Traditional Nigerian spices such as *Uda* (*Xylopia aethiopica*) and *Ehuru* (*Monodora myristica*) are frequently used for flavour enhancement and are culturally significant. Beyond their culinary use, they possess bioactive compounds with antimicrobial potential (Orji *et al.*, 2023). Phytochemical studies have shown that Ehuru is rich in terpenoids, alkaloids, flavonoids, and tannins with antimicrobial, antioxidant, and anti-inflammatory properties (Okata-Nwali & Uzoh, 2021). Uda, on the other hand, contains bioactive compounds such as glycosides, alkaloids, and saponins, with demonstrated antibacterial and antifungal activity (Ugoma *et al*., 2023). Traditionally, these spices are used in soups, stews, and meat preservation; however, scientific evidence supporting their role as natural preservatives in ready-to-eat foods, such as Abacha, remains limited. Previous studies have demonstrated that extracts of *Monodora myristica* and *Xylopia aethiopica* exhibit inhibitory effects against pathogens including *S. aureus*, *E. coli*, and *Salmonella spp*. (Enabulele *et al.*, 2014; Orji *et al*., 2023). Despite this, scientific literature on their application as natural preservatives in ready-to-eat street foods like *Abacha* remains limited. This study aims to investigate and compare the antimicrobial efficacy of *Uda* and *Ehuru* in *Abacha*, while also evaluating their sensory acceptability.

Materials and Methods

Sample Preparation

Sample Collection

Fresh samples of Abacha (African salad) were purchased from two different vendors within and around Godfrey Okoye University, Enugu. Each sample was collected in sterile, food-grade plastic containers, properly labeled (Vendor A and Vendor B), and immediately transported to the Biological Sciences Laboratory for analysis within one hour of purchase. Ehuru *(Monodora myristica)* and Uda *(Xylopia aethiopica)* seeds were also obtained from a local market in Enugu. The samples were placed in clean polythene bags, labeled accordingly, and taken to the laboratory for processing.

Preparation of Spice Powders

The Ehuru and Uda seeds were sorted to remove impurities, washed with distilled water, and air-dried at room temperature. The dried seeds were then ground into fine powders using a sanitized electric grinder. For treatment, 2 g and 5 g of each spice powder were weighed and thoroughly mixed into 100 g portions of the Abacha samples, corresponding to 2% and 5% concentrations, respectively. A sterile spatula was used to ensure uniform distribution of the spice powders throughout the samples (Okon *et al*., 2023).

Sample Division and Treatment

Both Abacha samples were divided into six portions and 100 grams were weighed out for each portion: first portion (plain Abacha for immediate analysis), second portion (Abacha mixed with 5% ehuru powder), third part portion (Abacha mixed with 2% ehuru powder), fourth portion (Abacha mixed with 5% Uda powder), fifth portion (Abacha mixed with 2% Uda powder) and last portion which was control (Plain Abacha without ehuru or uda). The concentrations were mixed thoroughly using aseptic technique and uniform distribution of ehuru/ uda throughout the Abacha was ensured. Portions 2-6 were then stored in sterile plastic containers with lids at room temperature for 4 hours before isolation of microorganisms (Ahmed *et al*., 2022; Zhang *et al*., 2016).

Determination of pH

The pH of Abacha samples was measured on arrival and after 4 hours of storage using a calibrated digital pH meter. The electrode was rinsed with distilled water and blotted dry before each measurement to avoid cross-contamination. The effect of Uda and Ehuru incorporation on pH was recorded to assess whether changes in acidity contributed to antimicrobial activity. (Khaizura et al., 2022).

Microbiological Analysis

Two-fold serial dilutions of the Abacha samples were prepared using sterile peptone water (Chauhan and Jindal, 2020). All culture media, including Nutrient Agar, MacConkey Agar, Mannitol Salt Agar, Eosin Methylene Blue Agar, and Salmonella-Shigella Agar, were prepared according to manufacturer's instructions. The media were sterilized by autoclaving at 121℃ for 15 minutes and allowed to cool before use. Aliquots from appropriate dilutions were inoculated onto the prepared agar plates using the pour-plate method. The plates were incubated at 37 ℃ for 24 hours to allow for microbial growth. Distinct colonies were isolated using sterile swabs and streaked onto fresh agar plates (MacConkey, Mannitol Salt Agar, and Salmonella-Shigella Agar) to obtain pure cultures. Plates were incubated at 37℃ for 24–48 hours. (Brown *et al.*, 2021).

Biochemical Characterization and Identification of Isolates

Gram Staining

Gram staining was used for preliminary classification of isolates. Smears were prepared, heat fixed, stained with crystal violet, treated with iodine, decolorized with alcohol and counterstained with safranin. Microscopic examination classified bacteria as Gram- positive (purple) or pink/red for Gram-negative (Tripathi and Sapra, 2022).

Catalase Test
A drop of 3% hydrogen peroxide was placed on a clean slide. A loopful of isolate was added and observed for bubble formation, indicating catalase-positive organisms (Mahon, 2011)

Coagulase Test
A bacterial suspension was prepared by mixing a loopful of the isolate with distilled water on a sterile dry slide. A drop of plasma was added to this suspension and mixed gently. The reaction was observed for coagulation within 10 seconds. This test was used to differentiate *Staphylococcus aureus* (coagulase - positive) from *Staphylococcus epidermidis* (coagulase - negative) (Beena, 2019).

Oxidase Test
Filter paper strips were moistened with oxidase reagent. A smear of bacterial isolate was added. A dark purple coloration within 30s indicated a positive result (Cheesebrough, 2006).

Indole Test
This test detects the ability of an isolate to decompose the amino acid tryptophan into indole using a colorimetric reaction with *p*-dimethylamino-benzaldehyde (Kovac's reagent). Peptone broth was prepared, dispensed into broth bottles and inoculated with the isolate. The inoculated broth was incubated at 37°C for 48 hours. After incubation, 5 to 7 drops of Kovac's reagent were gently added to each tube. A positive result was indicated by the formation of a red ring, while a negative result showed a yellow coloration (Aakanchha *et al*., 2020).

Citrate Utilization Test
Isolate was lightly inoculated on Simmons citrate agar slant and put in an incubator for 24-48 hours at 37°C. A positive result was demonstrated when there was a color change from green to blue; a negative result when no colour change was observed. The test was to determine whether or not an organism possesses the ability to utilize citrate as a sole carbon source and ammonium salts as a sole nitrogen source in growing (Cappuccino and Welsh, 2019).

Sensory Evaluation
A sensory evaluation of the treated and untreated Abacha samples was conducted using a panel of 20 semi-trained assessors, comprising staff and students from Godfrey Okoye University. The samples were coded to avoid bias and presented randomly in sterile disposable plates. Panelists rinsed their mouths with water between tastings. Each sample was evaluated for appearance, aroma, taste, texture, and overall acceptability using a 9-point hedonic scale, where 1 represented "extremely dislike" and 9 represented "extremely like." Mean scores for each attribute were calculated to determine consumer preference for the Uda- and Ehuru-treated Abacha samples.

Statistical Analysis
All analyses were performed in triplicate, and the results were expressed as means ± Standard Deviation. The obtained data were analyzed using one-way analysis of variance (ANOVA) as described by Iwe (2002). Mean values were separated using the Duncan test at a significance level of $P < 0.05$.

Results and Discussion

Microbial load and pH
The pH and microbial load of Abacha samples after 4 h of storage are presented in Table 1. Untreated Abacha showed microbial counts of $1.2 \times 10^3 – 1.6 \times 10^3$ CFU/mL, which increased to $2.5 \times 10^3 – 3.3 \times 10^3$ CFU/mL after 4 h storage. Ehuru significantly inhibited microbial growth, with 5% Ehuru reducing counts to 1.1×10^3 CFU/mL, approaching baseline levels. Uda also reduced microbial load but less effectively. pH values ranged from 6.75 to 7.25, with Uda-treated samples exhibiting slightly lower pH levels.

Table 1: pH and microbial load of Abacha samples after 4 h of storage

Sample	pH	Microbial count (CFU/ml
OAA	7.25 ± 0.002^{a}	$1.2x10^{3}\pm0.004^{b}$
CAO	7.05 ± 0.003^{c}	$2.5x10^{3}\pm0.005^{c}$
EAT	7.25 ± 0.011^{a}	$1.2x10^{3}\pm0.001^{b}$
EAF	7.15 ± 0.003^{b}	$1.1x10^{3}\pm0.015^{a}$
UAT	6.95 ± 0.015^{d}	$1.5x10^{3}\pm0.002^{bc}$
UAF	7.05 ± 0.001^{c}	$1.5x10^{3}\pm0.011^{bc}$
OBA	7.20 ± 0.010^{b}	$1.6x10^{3}\pm0.012^{c}$
CBO	6.95 ± 0.006^{d}	$3.3x10^{3}\pm0.021^{d}$
EBT	6.90 ± 0.021^{e}	$1.7x10^{3}\pm0.002^{c}$
EBF	7.15 ± 0.011^{b}	$1.2x10^{3}\pm0.030^{b}$
UBT	6.75 ± 0.003^{f}	$1.8x10^{3}\pm0.005^{c}$
UBF	6.90 ± 0.050^{e}	$1.5x10^{3}\pm0.001^{bc}$

OAA- On arrival samples from vendor A; OBA- On arrival samples from vendor B; CAO- Control without species from vendor A; EAT- Ehuru 2% from vendor A; EAF – Ehuru 5% from vendor A; EBT - Ehuru 2% from vendor B; EBF - Ehuru 5% from vendor B; CBO - Control without spices from vendor B; UAT - Uda 2% from vendor A; UAF - Uda 5% from vendor A; UBT - Uda 2% from vendor B; UBF - Uda 5% from vendor B

Identification of Isolates
Biochemical tests, as seen in Table 2, confirmed the presence of five pathogenic bacterial species in the Abacha samples: *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp*, *Pseudomonas aeruginosa* and *Enterobacter spp*. Vendor B samples contained all five organisms.

Table 2: Biochemical identification of microorganism isolated from abacha

Test	Bac 1	Bac 2	Bac 3	Bac 4	Bac 5
Shape	Cocci	Rod	Rod	Rod	Rod
Gram stain	+	-	_	-	-
Catalase	+	+	+	+	+
Oxidase	-	+	-	-	-
Indole	-	-	+	-	-
Citrate utilization	-	+	-	+	+
Coagulase	+	-	-	-	-
Probable organisms	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Salmonella spp.*	*Enterobacter spp*

(+) = positive and (-) = negative

Microbial Distribution between Vendors

Vendor A's Abacha (baseline) showed lower contamination, with only *S. aureus* and *E. coli* and isolate detected. Vendor B's Abacha, however, contained all five pathogens (Table 3), suggesting poorer hygiene during preparation or storage.

Table 3: Distribution of identified microorganisms in Abacha samples from vendor A and vendor B.

Sample	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Salmonella spp.*	*Enterobacter spp*
OAA	+	-	+	-	-
OBA	+	+	+	+	+

OAA- On arrival samples from vendor A; OBA- On arrival samples from vendor B; (+) indicating presence of microorganism and (-) indicating absence of microorganism.

Sensory evaluation

Sensory evaluation revealed significant differences in consumer acceptability between *Ehuru*- and *Uda*-treated *Abacha* samples (Figure 1). Overall, *Ehuru*-treated samples recorded substantially higher scores across all sensory attributes, including appearance, aroma, taste, texture, and overall consumer acceptability. Among these, the 2% *Ehuru* treatment from Vendor A (EAT) achieved the highest sensory ratings, with scores ranging from 7 to 8 on the 9-point hedonic scale, indicating strong consumer preference. Similarly, other *Ehuru*-treated samples (EAF, EBT, and EBF) maintained relatively high acceptability, with mean scores between 6 and 7 across most attributes. This suggests that *Ehuru* incorporation positively enhanced the sensory appeal of *Abacha*, likely due to its characteristic aromatic compounds and mild flavor profile, which complemented the product without overpowering it.

In contrast, *Uda*-treated samples were poorly rated across most sensory parameters. Especially taste and overall consumer acceptability received particularly low scores (1–2), indicating a strong dislike among panelists. Although aroma scores for *Uda*-treated samples were moderate (5–6), this did not translate into improved taste perception or acceptability, suggesting that the pungent or bitter flavor components of *Uda* adversely affected palatability. The reduced texture and appearance scores further contributed to the low overall acceptance of these samples.

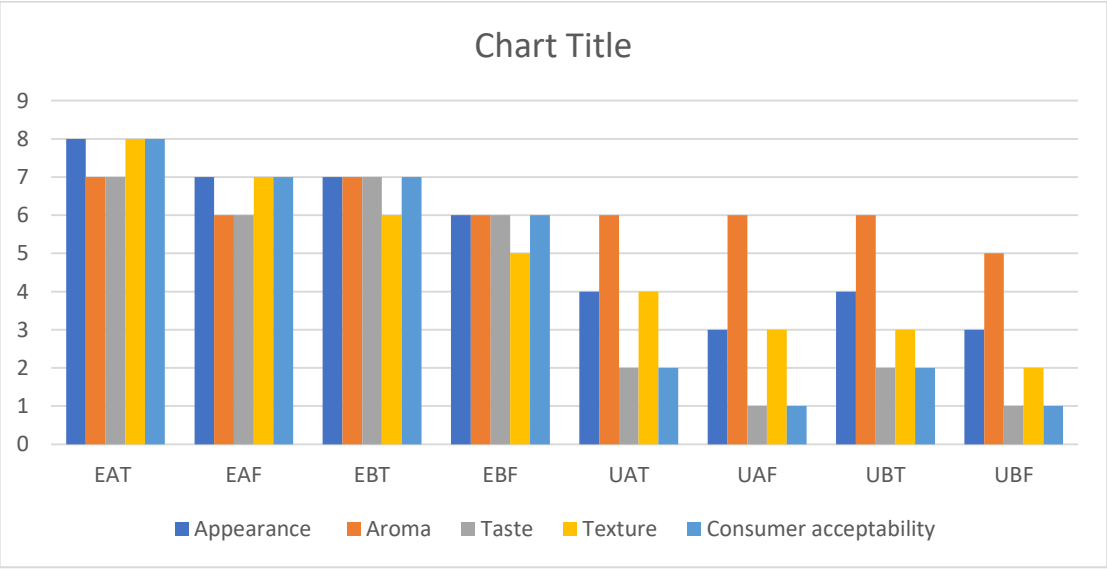

Figure 1: Sensory evaluation of the Abacha samples.

OAA- On arrival samples from vendor A; OBA- On arrival samples from vendor B; CAO- Control without species from vendor A; EAT- Ehuru 2% from vendor A; EAF – Ehuru 5% from vendor A; EBT - Ehuru 2% from vendor B; EBF - Ehuru 5% from vendor B; CBO - Control without spices from vendor B; UAT - Uda 2% from vendor A; UAF - Uda 5% from vendor A; UBT - Uda 2% from vendor B; UBF - Uda 5% from vendor B

Discussion

This study demonstrates that both *Monodora myristica* (Ehuru) and *Xylopia aethiopica* (Uda) possess antimicrobial activity against common foodborne pathogens in Abacha, but with significant differences in efficacy and consumer acceptance. The microbial analysis revealed the presence of *S. aureus*, *E. coli*, *Salmonella spp.*, *P. aeruginosa*, and *Enterobacter spp.*, confirming that Abacha is highly susceptible to contamination, particularly when prepared or stored under unhygienic conditions. These findings are consistent with previous reports identifying similar pathogens in Nigerian street foods (Eluu *et al.*, 2018; Osuji, 2024). The higher contamination levels observed in Vendor B's samples emphasize the role of vendor hygiene and preparation practices in determining microbial quality. Ehuru exhibited stronger antimicrobial activity than Uda, especially at 5% concentration, where microbial counts were reduced to near baseline levels. This aligns with phytochemical reports describing terpenoids, flavonoids, and alkaloids in Ehuru as potent antibacterial compounds (Okata-Nwali & Uzoh, 2021). In contrast, while Uda also reduced microbial load, its effect was weaker and appeared partially linked to pH reduction, suggesting acidification may contribute to its inhibitory action. These findings agree with previous studies on the antimicrobial effects of Uda in meat preservation and traditional soups (Tamfu *et al.*, 2020).

The sensory evaluation revealed a significant difference between the two spices. Ehuru-treated Abacha samples received significantly higher ratings for aroma, taste, and overall acceptability, particularly at 2% concentration. This suggests that Ehuru can serve a dual role as both a preservative and a flavour enhancer. Conversely, Uda-treated samples were poorly rated, with panelists noting an undesirable taste, limiting its practical application in ready-to-eat foods. Similar outcomes have been reported in sensory studies where high concentrations of Uda altered food palatability despite its antimicrobial properties (Eze *et al.*, 2021). The concentration-dependent effects observed in both spices highlight the need to optimize dosage in practical applications. While higher concentrations improve microbial inhibition, they may negatively impact sensory quality. Ehuru at 2–5% provides a balance between food safety and consumer preference, making it a more suitable candidate for commercial application in Abacha preservation.

Overall, this study supports the use of Ehuru as a natural preservative in Abacha, offering a culturally acceptable and safe alternative to synthetic preservatives such as sodium benzoate. However, the study was limited to short-term storage (4 h) and in vitro analysis. Future work should explore longer storage periods, synergistic effects with other spices, and in vivo safety assessments to validate these findings for broader food industry applications.

Conclusion

Ehuru *(Monodora myristica)* demonstrated superior antimicrobial efficacy and consumer acceptability compared to Uda *(Xylopia aethiopica*), establishing its potential as a natural preservative for Abacha. Promoting indigenous spices in food preservation could improve food safety in Nigeria while supporting sustainable and culturally relevant dietary practices.

Author Contributions

Chidinma A. Okafor**:** Conceptualization, Methodology, analysis and interpretation of data, review & editing, Ruth E. Ugwu and Favour E. Ogukah**:** Investigation and Original draft.

Data Availability Statement

Data will be made available on request.

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Ethical statement

The authors confirm that the corresponding protocols were followed for the correct protection of the rights and privacy of all participants, including no coercion to participate, and full disclosure of the study requirements and risks through the verbal consent of participants.

Declaration of Competing Interest

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the research reported in this paper.

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