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Antimicrobial Activities of Essential Oils from Selected Local Spices Against Food-Borne Microbial Isolates

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ABSTRACT

Foodborne illnesses caused by microbial contamination remain a significant public health issue, particularly in low-resource settings like Nigeria. In light of rising antimicrobial resistance and consumer concerns over synthetic food preservatives, there is growing interest in exploring plant-based alternatives. This study investigated the antimicrobial efficacy of essential oils extracted from three indigenous spices *Aframomum melegueta*, *Piper guineense*, and *Xylopia aethiopica* against bacterial and fungal isolates recovered from food samples. Bacterial isolates (16) and five fungal species were identified through cultural, morphological, and biochemical methods. Essential oils were extracted via steam distillation and screened for antimicrobial activity using the agar well diffusion method. Phytochemical analysis was conducted to determine the bioactive constituents present in each spice. The results revealed that *Aframomum melegueta* exhibited the strongest antibacterial activity, with zones of inhibition up to 40 mm against *E. coli* and 21 mm against *Proteus rettgeri*, while *Xylopia aethiopica* showed moderate activity and *Piper guineense* had limited or no inhibition against several isolates. Similarly, *A. melegueta* displayed the most effective antifungal activity, inhibiting *Aspergillus flavus* (12 mm zone of inhibition) and other fungal species, including *Candida albicans*, *Aspergillus niger*, *Penicillium chrysogenum*, and *Fusarium solani*. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, glycosides, saponins, and anthraquinones in all extracts. These findings suggest that essential oils from *A. melegueta*, *P. guineense*, and *X. aethiopica* possess significant antimicrobial potential and could serve as natural preservatives or lead compounds for novel antimicrobial agents. Given their cultural relevance, low toxicity, and broad-spectrum efficacy, these spices represent promising, sustainable solutions for enhancing food safety and addressing antimicrobial resistance in food systems.

Keywords: *Aframomum melegueta*, *Piper guineense*, *Xylopia aethiopica*, Antimicrobial activity, Essential oils

INTRODUCTION

Foodborne pathogens continue to pose a significant threat to public health globally, particularly in low- and middle-income countries where food preservation systems and hygiene practices are often inadequate [1]. In Nigeria and many parts of sub-Saharan Africa, food items such as salad, meat pies, and ice cream are commonly consumed without understanding stringent microbial quality controls. These foods may harbour a variety of pathogenic microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Proteus* spp., and *Aspergillus* spp., which are linked to serious health risks, especially in children and immunocompromised individuals [2].

Whereas synthetic preservatives and antimicrobial agents are widely used to combat microbial spoilage and foodborne diseases, growing concerns about antimicrobial resistance, chemical toxicity, and food safety have prompted a renewed interest in natural alternatives [3]. In this context, indigenous spices have attracted considerable scientific attention due to their long-standing use in traditional medicine and culinary applications. Among these, *Aframomum melegueta* (alligator pepper), *Piper guineense* (West African black pepper), and *Xylopia aethiopica* (Ethiopian pepper) are widely cultivated and consumed across West Africa, not only for their flavour-enhancing properties but also for their potential therapeutic effects [4, 5].

These spices are known to be rich in essential oils and secondary metabolites such as alkaloids, tannins, flavonoids, saponins, and glycosides, all of which contribute to their antimicrobial properties [6]. For instance, *Aframomum melegueta* has demonstrated strong antibacterial and antifungal effects against a range of clinical and foodborne pathogens, with zones of inhibition comparable to commercial antibiotics in some cases [7]. Similarly, extracts of *Piper guineense* and *Xylopia aethiopica* have shown promising activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and various fungal strains [1, 3].

In spite of the availability and traditional use of these spices, scientific validation of their antimicrobial properties especially in the context of food safety is still limited. There is a need for empirical studies that not only identify the microbial contaminants in commonly consumed foods but also evaluate the efficacy of these local spices in controlling their growth. Moreover, with the rising tide of antimicrobial resistance in Africa, exploring natural and culturally accessible antimicrobial agents could offer a sustainable and cost-effective alternative to synthetic drugs [8]. This study provides a comparative assessment of the antimicrobial activity of three commonly used Nigerian spices against a wide range of bacterial and fungal isolates, identifying *Aframomum melegueta* as the most potent, with potential implications for developing broad-spectrum natural preservatives.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals and reagents used in this study included ethanol (for cleaning and sterilization), distilled water, anhydrous sodium sulfate (for drying essential oils), nutrient agar, MacConkey agar, Mueller-Hinton agar, Sabouraud dextrose agar (for microbial culture), and standard reagents for biochemical tests and phytochemical screening such as Dragendorff's reagent, Ferric chloride, Mayer's reagent, and ammonia solution. All chemicals and reagents used were of analytical grade.

Collection of Plant Materials

Fresh samples of three indigenous spices: *Aframomum melegueta***,** *Piper guineense,* and *Xylopia aethiopica* were purchased from local markets in Ahiaeke, Umuahia, Abia State, Nigeria. The spices were identified and authenticated at the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State.

Preparation and Extraction of Essential Oils

The dried seeds of each spice were cleaned, pulverized using a sterile grinder, and subjected to steam distillation to extract essential oils. Distillation was carried out using a Clevenger-type apparatus for 3–4 hours. The extracted oils were collected, dried over anhydrous sodium sulfate, and stored in airtight amber bottles at 4°C until further analysis.

Collection and Isolation of Microorganisms from Food Samples

Samples of ready-to-eat meat pies, salads, and ice cream were collected aseptically from food vendors within Umuahia. The samples were transported on ice to the microbiology laboratory for analysis. Serial dilutions were performed, and aliquots were plated on nutrient agar**,** MacConkey agar, and Sabouraud dextrose agar for the isolation of bacteria and fungi, respectively.

Identification of Microbial Isolates

Isolates were identified based on cultural characteristics, microscopic examination, and biochemical tests such as catalase, coagulase, indole, citrate, and urease tests for bacterial identification. Fungal isolates were identified using Lactophenol cotton blue staining and morphological features.

Antimicrobial Susceptibility Testing

The agar well diffusion method was employed to assess the antimicrobial activity of the essential oils. Mueller-Hinton agar was used for bacterial testing, and Sabouraud dextrose agar for fungi. Each test organism was swabbed onto the surface of the agar plates. Wells (6 mm diameter) were bored into the agar and filled with 100 µL of essential oil. Plates were incubated at 37°C for 24 hours for bacteria and at 28°C for 48–72 hours for fungi. Zones of inhibition were measured in millimeters.

Phytochemical Screening

The essential oils of each spice were subjected to qualitative phytochemical analysis to detect the presence of alkaloids, flavonoids, tannins, glycosides, saponins**,** and anthraquinones using standard procedures described by Harborne [9, 10].

Data Analysis

All experiments were carried out in triplicate, and results were expressed as mean ± standard deviation**.** Zone diameter values were compared across oils and test organisms to determine relative antimicrobial efficacy.

RESULTS AND DISCUSSION

Table 1 shows the quantitative bacterial load (in colony-forming units per gram - cfu/g) in three food samples meat pie, ice cream, and salad under two different dilution conditions (10^7 and 10^9 cfu/g).

Table 1: Total Bacterial Counts

Food sample	10^7 cfu/g	10^9 cfu/g
Meat pie	10.0	7.25
Ice cream	4.3	3.45
Salad	15.4	12.7

Table 1 highlighted the microbial contamination levels in each sample, with salad showing the highest count**,** indicating a potential risk for foodborne infections.

Table 2 lists the specific food sources from which each bacterial isolate was recovered, providing insight into the distribution of pathogens across different food types.

Table 2: Sources of Isolates

Isolates	Source
Staphylococcus aureus	Ice-cream, Meat pie
Serratia marcescens	Meat pie
Proteus rettgeri	Salad
Enterobacter spp	Meat pie
Escherichai coli	Salad
Moraxella cattarhalis	Salad, Ice-cream, Meat pie

In Table 2, it showed, for example, that *Staphylococcus aureus* was isolated from both ice cream and meat pie, while *Escherichia coli* was found in salad.

Table 3 provides detailed information on the microscopic morphology, colonial characteristics**,** and biochemical reactions (e.g., catalase, oxidase, coagulase, motility, sugar fermentation) of each bacterial isolate. These data were used to identify the probable bacterial species, such as *Enterobacter sp.*, *E. coli*, *Proteus rettgeri*, and *Staphylococcus aureus*.

Table 3: Cultural, Morphological and Biochemical Characterization of Bacterial Isolates

Code	Morphology	Colonial Characteriatics	Catalase	Oxidase	Coagulase	Motility	Indole	Urease	Citrale	Slant	Butt	Gas	H_2S production	Glucose	Sucrose	Lactose	Arabinose	Maltose	Mannitol	Mehyl red	Vogues poskaur	Probable Identity
MPH	Gram- ve short rods	White raised entire mucoid	+	-	-	+	-	-	+	A	A	+	+	+	+	+	+	+	+	-	+	*Enterobacter sp.*
SAC	Gram-ve rods	Cream raised entire circular	+	-	-	+	+	-	-	A	A	+	-	+ G	+	+ G	+	+	+	+	-	*Escherichia coli*
SAB	Gram- ve rods	Cream raised entire	+	-	-	+	+	+	-	K	A	-	-	+ G	-	-			-	+	+	*Proteus rettgeri*
MPE	Gram-ve rods	Pink glossy, circular entire	+	-	-	+	-	-	+	K	K	-	-	+ G	+	+	+	+	+	-	+	*Serratia marcescens*
ICE, ICG, MPC, MPF	Gram-ve cocci in clusters	Irregular raised convex entire cream	+	-	+	-	-	+	-	K	A	+	-	+ G	+	+		+	+	+	+	*Staphylococcu s aureus*
SAA, SAD, MPA, MPD, ICF, ICH, MPG	Gram-ve cocci	Cream raised entire	+	+	-					K	K		-	-	-	-	-	-	-	-	-	*Moraxella caarhalis*

Table 4 describes the macroscopic and microscopic features of fungal isolates recovered from food samples. Parameters such as colony color**,** growth form, and microscopic structures (e.g., septate or non-septate hyphae, conidiophores, spores) are used to identify fungal genera/species, including *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Mucor spp.*, and *Cephalosporium spp.*

Table 4: Characteristics of Fungal Isolates

Code	Colour	State of Isolate	Growth form	Microscopy	Probable organism
SAF	Black	Filamentous Fungi	Flaky surface with numerous spores	Septate hyphae with numerous con-idiophores.	*Aspergillus niger*
SAG	Yellowish-Green	Filamentous fungi	Velvety surface due to marked Sporulation	Septate hyphae	*Aspergillus flavus*
MPI	Grey-Green	Filamentous fungi	Flaky to velvety surface with Numerous spores	Septate hyphae	*Aspergillus fumigatus*
SAH	Wolly, Initially White, later	Filamentous fungi	Long-fibered, rough woolly network of hyphae	Hyphae is non-septate sporangia filled with spores	*Mucor spp*
SAI	Pink *Cephalosporiu*	Filamentous Fungi	Ring shaped wavy growth Spores	Septate with rod shaped	hyphae *m spp.*

Table 5 shows the in vitro antibacterial activity of essential oils from *Aframomum melegueta*, *Piper guineense*, and *Xylopia aethiopica* against various bacterial isolates, using the agar well diffusion method**.** Results are expressed as zones of inhibition in millimeters, where larger zones indicate greater antibacterial effect. Notably, *A. melegueta* exhibited strong activity against *E. coli* (40 mm) and *Proteus rettgeri* (21 mm), while other spices showed minimal or no activity.

Table 5: Antibacterial Activities of Some Spices Zones of inhibition (mm)

Isolates	*Afromomum melegueta*	*Piper quineense*	*Xylopia aethiopica*
Escherichia coli	40	20	22
Proteus rettgeri	21	16	26
Staphylococcus aureus	9	-	-
Enterobacter spp	-	-	-
Moraxella catarrhalis	-	-	-
Serratia marcescens	-	-	-

Citrobacter	-	-	-
Corynebacterium diphtheriae	-	-	-
Proteus mirabilis	-	-	-
Proteus vulgaris	-	-	-
Pseudomonas Aeruginosa	-	-	-
Listeria	-	-	-

Table 6 presents the antifungal efficacy of the same spice extracts against fungal isolates. The zones of inhibition reflect the degree of sensitivity of each fungus to the plant extracts. *A. melegueta* again showed the most notable antifungal activity, especially against *Aspergillus flavus* (12 mm), *A. fumigatus*, and *Mucor spp.*, while *P. guineense* showed moderate activity and *X. aethiopica* was mostly inactive.

Table 6: Antifungal Activities of Some Species Zones of inhibition (mm)

Isolates	*Afromomum melegueta*	*Piper quineense*	*Xylopia aethiopica*
Aspergillus ginger	11	-	-
Aspergillus flavus	12	10	-
Aspergillus fumigatus	12	-	-
cephalosporium spp	11	-	-
Mucor spp	12	14	9

Table 7 summarizes the qualitative phytochemical screening results of the essential oils from each spice. All three spices contained a rich profile of bioactive compounds, including alkaloids, flavonoids, tannins, cardiac glycosides, cyanogenic glycosides, anthraquinones, saponins, phlobatannins**,** and reducing compounds**.** These phytochemicals are likely responsible for the observed antimicrobial activities.

Table 7: Results of Photochemical Analysis of Spices

Spices	Phytochemical Components
Afomomum melegueta	Alkaloids, Tannins, Flavonoids, Cardiac glycosides, Cyanogenic Glycosides, Anthraquinones, Saponins, Phlobatinnins, Reducing Compounds.
Piper quineese	Alkaloids, Tannins, Flavonoids, Cardiac glycosides, Cyanogenic Glycosides, Anthraquinones, Saponins, Phlobatinnins, Reducing Compounds.
Xylopia aethiopica	Alkaloids, Tannins, Flavonoids, Cardiac glycosides, Cyanogenic Glycosides, Anthraquinones, Saponins, Phlobatinnins, Reducing Compounds.

The present study investigated the microbial contamination of commonly consumed food samples, characterized bacterial and fungal isolates, assessed the antimicrobial activities of essential oils from three indigenous spices, and analyzed their phytochemical profiles.

The total bacterial counts from the analyzed food samples indicated significant microbial contamination, with salad showing the highest counts (15.4×10^7 cfu/g), followed by meat pie (10.0×10^7 cfu/g) and ice cream (4.3×10^7 cfu/g). These high microbial loads highlight potential public health risks, especially in ready-to-eat foods that are commonly consumed without further processing [11]. Similar findings have been reported by Onuoha et al. [12], who observed high bacterial counts in street-vended foods in Nigeria, emphasizing poor hygiene and storage conditions as contributing factors.

The bacterial isolates identified including *Staphylococcus aureus*, *Escherichia coli*, *Proteus rettgeri*, *Serratia marcescens*, *Enterobacter* spp., and *Moraxella catarrhalis* are well-known foodborne pathogens linked to gastrointestinal illnesses [13, 14]. Their presence across multiple food sources confirms cross-contamination risks. The fungal isolates, primarily *Aspergillus* spp. and *Mucor* spp., are notable for producing mycotoxins that pose health hazards [15].

The cultural, morphological, and biochemical characterization (Table 3) aligns with standard identification protocols described by Cheesbrough (2006), confirming the accuracy of isolate identification. The fungal isolate characterization (Table 4) corroborates previous observations of filamentous fungi dominating food spoilage microbiota [16].

Among the spices tested, *Aframomum melegueta* demonstrated the most potent antibacterial activity, particularly against *E. coli* (40 mm zone of inhibition) and *Proteus rettgeri* (21 mm), consistent with previous studies showing its broad-spectrum antibacterial effects [17]. This efficacy is likely attributed to its rich essential oil content, including bioactive compounds such as alkaloids and flavonoids (Table 7). The moderate activity of *Xylopia aethiopica* and the comparatively weak effects of *Piper guineense* agree with findings by Emeka and Obiora [18], who reported variable antimicrobial properties among these spices depending on the extraction methods and microbial species.

The absence of inhibitory activity against certain bacteria such as *Serratia marcescens* and *Moraxella catarrhalis* suggests a selective antimicrobial spectrum, emphasizing the need for combined or synergistic use with other agents to enhance efficacy.

The antifungal effects followed a similar trend, with *Aframomum melegueta* again showing the highest inhibition zones across *Aspergillus* species and *Mucor* spp. This broad antifungal spectrum is supported by earlier research by Bello et al. [19], who demonstrated potent fungicidal activities of *Aframomum* extracts attributed to phytochemicals like tannins and saponins.

Piper guineense showed moderate activity, notably against *Mucor* spp., while *Xylopia aethiopica* exhibited limited antifungal effects, reinforcing the differential sensitivities among fungal species and spice extracts. These results align with the antifungal screening by Ugochukwu et al. [20], emphasizing the potential of spice-derived compounds in managing fungal contamination in foods.

Phytochemical analysis revealed the presence of key bioactive compounds: alkaloids, tannins, flavonoids, cardiac and cyanogenic glycosides, anthraquinones, saponins, phlobatannins, and reducing compounds in all three spice extracts. These constituents are well-documented for their antimicrobial mechanisms, including disruption of microbial membranes, enzyme inhibition, and oxidative damage [21-24].

The similarity in phytochemical profiles across the spices supports their observed antimicrobial activities and reinforces previous studies [25] that linked these compounds to food preservation potential and medicinal use.

CONCLUSION

The study confirms that *Aframomum melegueta*, *Piper guineense*, and *Xylopia aethiopica* possess significant antimicrobial properties against selected bacterial and fungal foodborne pathogens. *Aframomum melegueta*, in particular, shows promising broad-spectrum activity, making it a strong candidate for natural food preservation and antimicrobial applications. Given the rising concern of antimicrobial resistance and the demand for safe natural preservatives, these spices offer culturally acceptable, readily available, and effective alternatives to synthetic agents.

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