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Inhibitory Potential of Native Spices Against Fruit-borne Environmental Microrganisms

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ABSTRACT

This study investigated the antimicrobial potential of native spices against environmental pathogens isolated from ready-to-eat fruits sold in Port Harcourt Metropolis. A total of 10 different native spices were evaluated, including ginger, cinnamon, turmeric, clove, Negro pepper, and Aidan fruit. Pathogenic bacterial strains were isolated and identified from 15 ready-to-eat fruit samples (watermelon, pineapple, and pawpaw) collected from various vendors across Port Harcourt Metropolis. The study evaluated the antibacterial properties of these spices against the isolated microbial contaminants and compared their effectiveness in inhibiting microbial growth. The antimicrobial activity of each spice extract was measured using the disc diffusion method. The results indicated that all 10 spices exhibited varying levels of antimicrobial activity against the pathogens, with cinnamon and nutmeg demonstrating the highest inhibitory effects, producing zones of inhibition ranging from 15 to 25 mm. These findings demonstrate the potential of native spices as natural alternatives to synthetic antimicrobial agents for controlling environmental pathogens in ready-to-eat fruits essential for food safety application.

KEYWORDS: Antimicrobial, Native Spices, Clove, Environmental Pathogens, Ready-to-Eat Fruits, Food Safety.

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1. Introduction

Fruits are among the most widely consumed food items in the world because they are nutritious, refreshing, and provide essential vitamins, minerals, and antioxidants necessary for human health (Abobatta, 2021). In particular, ready-to-eat fruits such as watermelon (Citrullus lanatus), pineapple (Ananas comosus), and pawpaw (Carica papaya) are very popular in Nigeria due to their sweet taste, affordable cost, and high water content which helps in hydration (Uthman & Garba, 2023). They are usually eaten fresh without further cooking, making them convenient sources of nutrition for both adults and children. However, this convenience also makes them highly vulnerable to microbial contamination, as they are often exposed to unhygienic handling, contaminated water, poor packaging, and unsanitary market conditions (Kalpana et al., 2024).

The increasing consumption of ready-to-eat fruits in urban areas such as Port Harcourt comes with food safety challenges. Several studies have reported that fruits can serve as carriers of dangerous microbes such Staphylococcus aureus, Salmonella spp., Escherichia coli, and Aspergillus spp., which cause foodborne infections (RK, 2022). These microbial contaminants often originate from the soil, irrigation water, fruit handlers, or contaminated storage environments (Wallace et al., 2020). Since Fruits that are ready to eat are consumed raw, the risk of infection is heightened because the microorganisms are not destroyed by heat treatment, as would be the case in cooked foods (Machado-Moreira et al., 2019). Outbreaks of foodborne diseases have been linked to contaminated fruits, and this has raised public health concerns, especially in

developing countries where food safety regulations are not strictly enforced (Subedi et al., 2025).

The common approach to controlling microbial contamination in foods is the use of chemical preservatives and antibiotics (Melo and Quintas, 2023). However, these have several drawbacks. Chemical preservatives may leave harmful residues in food, which can accumulate in the human body and cause long-term health problems such as carcinogenic effects and organ toxicity (Bereda, 2025). Additionally, the excessive use of synthetic antibiotics in food preservation and medical treatment has contributed to the growing problem of antimicrobial resistance (Kobets et al., 2022).

As a result of these challenges, attention has shifted towards safer, natural, and eco-friendly alternatives to synthetic chemicals. Plants, particularly spices, have attracted great interest because of their antimicrobial properties that are linked to their bioactive phytochemicals (Murugan, 2022). Spices are widely used in traditional medicine and food preservation, and their effectiveness against bacteria and fungi has been scientifically established. Spices with great potential includes clove (Syzygium aromaticum), Negro pepper (Xylopia aethiopica), and Aidan fruit (Tetrapleura tetraptera). These spices are common in West Africa, including Nigeria, where they are valued not only for flavoring food but also for their medicinal benefits (Xedzro et al., 2022).

Clove is rich in eugenol, an essential oil compound that has strong antimicrobial, antifungal, and antioxidant properties (Uhuo et al., 2024). Negro pepper contains alkaloids, terpenoids, and flavonoids (Ahmad Wani et al., 2022) that contribute to its traditional use in treating respiratory and gastrointestinal infections. Aidan fruit is widely recognized for its health-promoting effects and contains saponins, tannins, and phenolic compounds that have antimicrobial potential (Uhuo et al., 2024). The use of these spices as natural antimicrobial agents could provide a sustainable and safe way to reduce microbial contamination in ready-to-eat fruits.

In Port Harcourt metropolis, where fresh fruits are sold daily in open markets, roadside stands, and supermarkets, microbial contamination poses a constant public health concern. Assessing the antimicrobial potential of clove, Negro pepper, and Aidan fruit against microbial contaminants isolated from commonly consumed fruits such as watermelon, pineapple, and pawpaw is therefore highly relevant. The outcome of this research did not only contribute to food safety knowledge but also promote the use of native spices as natural, affordable, and effective alternatives to synthetic preservatives in Nigeria.

Materials and Methods

Sample Collection, Preparation and Serial Dilution

A total of 15 ready-to-eat (RTE) fruit samples—comprising watermelon, pineapple, and pawpaw (n = 5 per fruit type), were randomly collected from various local vendors across Port Harcourt Metropolis, Rivers State, Nigeria. Samples were placed in sterile polyethylene bags, maintained in ice-packed transport containers at ~4°C, and transferred immediately to the laboratory for microbiological analysis within 2 hours of collection.

Isolation and Identification of Pathogenic Bacteria

Fruit samples were processed under aseptic conditions. A 10 g portion of each homogenized sample was transferred into 90 mL of sterile normal saline to prepare initial stock suspensions. Serial tenfold dilutions were subsequently performed from 10-1 to 106.

Inoculation and Incubation

Aliquots (0.1 mL) from the 10-1 and10-2 dilutions were inoculated in duplicate onto pre-poured, sterile Nutrient Agar (NA) plates for total heterotrophic bacterial counts (THBC), and Sabouraud Dextrose Agar (SDA) for fungal counts. NA plates were incubated at 37oC for 24 to 48 hours, while SDA plates at ambient temperature (28±2) for 3 to 5 days.

Isolated bacterial colonies were purified by subculturing onto fresh Nutrient Agar plates. Pathogenic bacterial strains were identified based on morphological characteristics, Gram staining, and standard biochemical tests (including catalase, oxidase, citrate, indole, citrate utilization, urease, and sugar fermentation profiles including glucose, sucrose, maltose and mannitol).

Isolation and Identification of Fungi

Aliquots (0.1 mL) of appropriate serial dilutions (10-2 to 10-4) were spread-plated onto Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (100 mg/L) to suppress bacterial growth. The culture plates were incubated at room temperature (28 ± 2°C) for 3 to 5 days.

Distinct fungal colonies (moulds and yeasts) were subcultured onto fresh SDA plates to obtain pure cultures. Isolates were characterized macroscopically by colony colour, texture, shape, surface appearance, and growth rate. Microscopic identification was performed using needle/wet mount preparations stained with Lactophenol Cotton Blue (LPCB) to evaluate vegetative mycelia (septation), reproductive structures (sporangia, conidial heads, arthrospores), hyphal organization, and spore morphology against standard mycological keys (Shanahan, 2024; Olumuyiwa et al., 2025).

Selection and Extraction of Native Spices

Ten native spice species—including ginger (Zingiber officinale), cinnamon (Cinnamomum verum), turmeric (Curcuma longa), clove (Syzygium aromaticum), Negro pepper (Xylopia aethiopica), and Aidan fruit (Tetrapleura tetraptera)—were sourced from local markets in Port Harcourt. The plant materials were thoroughly washed with distilled water, shade-dried at room temperature to a constant weight, and pulverized into a fine powder using a sterile grinder/mortar and pestle.

Extractions were performed using 85% ethanol and sterile distilled water as solvents. For each extraction, 50 g of pulverized spice powder was macerated in 250 mL of the respective solvent (1:5 w/v ratio) for 72 hours at room temperature with periodic agitation. The mixtures were filtered through Whatman No. 1 filter paper. Aqueous extracts were lyophilized, while ethanolic filtrates were concentrated to dryness under reduced pressure at 40°C using a rotary eva8orator. The resulting crude extracts were dissolved in 85% ethanol (for ethanolic extracts) or sterile distilled water (for aqueous extracts) to yield stock solutions, which were subsequently diluted to test concentrations of 50, 100, 200, and 400 mg/mL. Extracts were stored at 4°C until evaluation.

Test Microorganisms and Inoculum Standardization

The test microorganisms comprised confirmed pathogenic bacterial isolates—Gram-positive (Bacillus sp. and Staphylococcus sp.) and Gram-negative (Salmonella sp.)—as well as fungal isolates (Saccharomyces sp., Penicillium sp., and Aspergillus niger) (Luca et al., 2024). Isolates were identified using macroscopic/microscopic traits and biochemical characterization (including catalase, oxidase, motility, citrate utilization, salt tolerance, starch hydrolysis, indole production, and MR-VP assays).

Prior to susceptibility testing, inocula were standardized to ensure uniform cell density:

Bacterial Inoculum Preparation:

To prepare a standardized bacterial inoculum, 3 to 5 morphologically identical, actively growing colonies from a pure 18- to 24-hour agar culture were picked with a sterile loop and suspended in a sterile normal saline (0.85% NaCl), The resulting suspension was standardized by adjusting its turbidity to match a 0.5 McFarland standard (1.5 x 108 CFU/mL) confirmed spectrophotometrically (OD600=0.08-0.10). Finally, the suspension was serially diluted in saline to obtain the desired working concentration and used within 15 to 30 minutes to ensure cell density and viability remain stable (Hulankova, 2024).

Fungal Inoculum: For yeast (Saccharomyces sp.), suspensions were similarly adjusted to a 0.5 McFarland standard (1 x 106 to 5 x 106 cells/mL) (For filamentous fungi (Penicillium sp. and A. niger), spore suspensions were harvested from 5–7day old Potato Dextrose Broth or SDA cultures using sterile physiological saline containing 0.05% Tween 80 and adjusted to approximately 1 x 106 spores/mL using a hemocytometer (Boro & Narzary, 2022).

Antimicrobial Susceptibility Testing

Antimicrobial activity was evaluated using the agar well diffusion method (Provine & Hadley, 2000). Mueller-Hinton Agar (or Nutrient Agar) plates were prepared for bacterial testing, and Sabouraud Dextrose Agar (or Potato Dextrose Agar) plates were prepared for fungal testing. A sterile cotton swab was dipped into the

standardized microbial suspension and uniformly spread across the agar surface to create a lawn.

Wells of 4–6 mm diameter were bored into the inoculated media using a sterile cork borer. Aliquots (100 µL) of the spice extracts at various concentrations were loaded into designated wells. Ciprofloxacin (for bacteria) and ketoconazole (for fungi) served as positive controls, while pure extraction solvents (85% ethanol and sterile distilled water) served as negative controls.

Plates were left at room temperature for 1 hour to allow extract pre-diffusion, then incubated at 37°C for 24–48 hours for bacteria and at 28 ± 2°C for 3–5 days for fungi. Following incubation, antimicrobial efficacy was quantified by measuring clear zones of inhibition around each well in millimeters using a transparent ruler or Vernier caliper (Gajic et al., 2022). All assays were performed in triplicate.

Results and Discussion

Table 1 presents the results of microbial population of bacterial and fungal isolates. Across all fruit samples tested, bacterial counts were consistently higher than fungal counts. Total heterotrophic bacterial populations ranged from 3.53 to 3.91 $\log_{10}$ CFU/ml, with pineapple harbouring the highest concentration at 3.91 ± 0.00 $\log_{10}$ CFU/ml, followed by pawpaw at 3.60 ± 0.00 $\log_{10}$ CFU/ml and watermelon at 3.53 ± 0.00 $\log_{10}$ CFU/ml. Conversely, total heterotrophic fungal populations were lower, ranging from 2.59 to 2.98 $\log_{10}$ CFU/ml. Watermelon recorded the highest fungal load at 2.98 ± 0.70 $\log_{10}$ CFU/ml, while pineapple and pawpaw followed with 2.86 ± 0.00 $\log_{10}$ CFU/ml and 2.59 ± 0.00 $\log_{10}$ CFU/ml, respectively. Bacteria predominated over fungi in every sample, exceeding fungal counts by 0.55 $\log_{10}$ CFU/ml in watermelon, 1.01 $\log_{10}$ CFU/ml in pawpaw, and 1.05 $\log_{10}$ CFU/ml in pineapple. However, because all values share the same superscript, these variations across the different fruits are not statistically significantly different (p>0.05).

Table 1: Microbial Population of Bacterial and Fungal Isolates

SAMPLE	THB ($\log_{10}$CFU/ml)	THF ($\log_{10}$CFU/ml)
Water Melon	3.53^{a}±0.00*	2.98^{a}±0.70
Pineapple	3.91^{a}±0.00	2.86^{a}±0.00
Pawpaw	3.60^{a}±0.00	2.59^{a}±0.00

Key: *Values are means of duplicate determination. Means with the same superscript alphabets in the same column are not significantly different (p>0.05); THB-Total Heterotrophic Bacteria; THF - Total Heterotrophic Fungi; log10 CFU/ml.

The mean populations of Total Heterotrophic Bacteria (THB) and Total Heterotrophic Fungi (THF) isolated from three fruits—Pawpaw, Pineapple, and Watermelon shows no statistically significant variation. In relation to total heterotrophic bacteria (THB), pineapple (PA) had the highest mean bacterial count (3.91±0.00 log10 CFU/ml). Pawpaw (PP) came next with 3.60±0.00 log10 CFU/ml. Watermelon (WL) showcased the lowest bacterial burden at 3.53±0.00 log10 CFU/ml. Total Heterotrophic Fungi (THF) showed that watermelon (WL) had the highest fungal population (2.98±0.70 log10 CFU/ml). Next was pineapple (PA), which had 2.86±0.00 log10 CFU/ml. THB and THF loading varied among watermelon, pineapple, and pawpaw (p>0.05). The microbial loads seen in this data are in line with the pattern documented in tropical food literature on microbiology and the results of (Okoronkwo & Disegha, 2020) who assessed ready-to-eat fruits in southern Nigeria. and found that pineapple had a higher total bacterial count (1.06×105 log10 CFU/g) than watermelon (3.95×104 log10 CFU/g), which is consistent with the slightly higher bacterial count in pineapple (3.91 log10 CFU/ml). A research by (Wondimagegn & Mohammed, 2025) on unpasteurized fruit juices revealed a baseline aerobic mesophilic counts between 3.0 and 4.5 log10 CFU/ml which is comparable to the overall bacterial levels across all three fruits (ranging from 3.53 to 3.91 log10 CFU/ml). Watermelon yielding the highest fungal count (2.98 log10 CFU/ml) matches observations by (Ike et al., 2020). Given that watermelons grow directly on the ground, soil-dwelling moulds and yeasts (including Aspergillus, Fusarium, and Penicillium species) can easily migrate to the inner flesh during cutting or processing if cross-contamination takes place (Gu et al., 2022).

The Food and Agriculture Organization (FAO) and the International Commission on Microbiological Specifications for Foods (ICMSF) have established food safety guidelines that state that aerobic bacterial counts in fresh juices or ready-to-eat foods should not exceed 1.0×104 CFU/ml, or 4.0 log10 CFU/ml (Oranusi et al., 2012). The THB values in this data (3.53 to 3.91 log10 CFU/ml) are approaching the maximum allowable

limit of microbiological quality level. While they do not explicitly exceed the limit, they represent slight risk on the health of consumers (Tamiru et al., 2024).

The fungal populations (2.59 to 2.98 log10 CFU/ml) have substantial implications for the shelf-life of these fruits or their extracted juices. These fruits, particularly pineapple, are great substrates for acid-tolerant yeasts and moulds because they have low pH profiles and large concentrations of fermentable sugars. These fungal loads will quickly deteriorate if left untreated, resulting in obvious structural decay, cloud loss, and off flavours from the fermentation of alcoholic or organic acids (Omorodion and Osakuade, 2026).

The microbial populations of these several fruits do not differ significantly ($p>0.05$), which clearly suggests a shared source of contamination (MacArthur et al., 2021). The consistent microbial loading suggests systemic hygiene failures rather than fruit-specific problems because these samples were bought from street vendors or processed using shared tools.

This points to external vectors such as contaminated water, poor sanitization of knives, cutting boards, inadequate personal hygiene or handwashing practices by handlers. The existence of heterotrophic communities at these levels suggests that pathogenic strains, such as Staphylococcus aureus, Salmonella species, or Escherichia coli, might readily coexist with them, opening the door to foodborne diseases (Dembele et al., 2025).

identification to the species level.

Table 2 presents results of biochemical and morphological profiling of the nine samples which yielded a diverse population composed of both Gram-positive and Gram-negative taxa. Gram-positive bacteria accounted for the majority of strains (66.7%), segregated into cocci (Micrococcus, Staphylococcus, Enterococcus) and spore-forming or anaerobic rods (Bacillus, Clostridium). Gram-negative bacilli comprised enteric representatives matching phenotypic criteria for Salmonella and Escherichia coli.

The phenotypic characterization aligns with classical diagnostic parameters described in diagnostic bacteriology literature (Hulankova, 2024). Isolates from sample 1 and 2 were identified as Micrococcus and Staphylococcus species, supported by their Gram-positive coccoid morphology, positive catalase and oxidase (for Micrococcus) reactions, and fermentative capabilities.

Among the rod-shaped isolates, Isolate 3 exhibited catalase-negative and obligate anaerobic traits consistent with Clostridium species (Mustafina et al., 2024), whereas Isolates 4 and 8 demonstrated profiles characteristic of Bacillus species, highlighted by positive catalase, oxidase, and starch hydrolysis results (Olateru et al., 2025).

Enteric organisms were isolated from samples 5 and 6. Isolates from sample 6 displayed typical coliform traits including a positive methyl red, positive indole reaction, and characteristic acid-and-gas (AG) carbohydrate fermentation patterns diagnostic of Escherichia coli (Ema et al., 2022). Isolates from sample 5 demonstrated typical non-lactose fermenting bacterial behavior alongside motility and positive methyl red/indole reactions, matching general diagnostic markers for Salmonella species. The simultaneous recovery of environmental saprophytes (Bacillus and Micrococcus species) alongside faecal coliforms and enteric pathogens (Escherichia coli and Salmonella species) indicates a dual contamination pathway involving both soil-borne detrital inputs and faecal-oral transmission vectors. Definitive strain-level identification and source attribution will necessitate downstream molecular characterization via 16S rRNA gene sequencing.

Isolate	MMP	CAT	OXI	SH	CIT	ST	URS	UP	MR	IND	MOT	GLU	MAN	LAC	MAL	XY	Organism Identity
1 WL	GPC	+	+	-	-	+	-	+	-	-	-	A	A	A	A	A	*Micrococcus* sp
2 WL	GPC	+	+	-	-	+	-	+	-	-	-	A	A	A	A	A	*Staphylococcus* sp
3 PA	GPR	-	-	-	+	-	-	-	+	-	+	A	A	N	A	A	*Clostridium* sp
4 PA	GPR	+	+	+	+	+	-	-	+	-	+	A	A	N	A	A	*Bacillus* sp
5 PA	GNR	+	-	-	-	-	-	-	+	+	+	A	A	N	A	N	*Salmonella* sp

6 PP	GNR	+	-	-	+	-	+	-	+	+	+	AG	A	AG	A	A	*E. coli* sp
7 PP	GNC	+	-	-	+	-	-	+	-	-	+	N	A	AG	A	A	*Enterococcus*
8 PP	GPR	+	+	+	+	-	-	-	+	-	+	A	A	N	A	A	*Bacillus* sp

Table 2: Biochemical Test and Sugar Fermentation of the Test Isolates.

Key: WL = Watermelon samples; OXI = Oxidase; ;MAN = Mannitol; PA = Pineapple samples; SH = Starch Hydrolysis; LAC = Lactose; PP = Paw-paw samples; CIT = Citrate; MAL = Maltose; ISC = Isolate Code; ST = Salt Tolerance; XY = Xylose; MMP = Microbial Morphology; URS = Urease; A/G = Acid/Gas; GPC = Gram Positive Cocci; VP = Voges Proskauer; A = Acid; GPR = Gram Positive Rod; MR = Methyl Red; G = Gas; GNR = Gram Negative Rod; IND = Indole; N = No Acid/Gas; GNC = Gram Negative Cocci; MOT = Motility; + = Positive; CAT = Catalase; GLU = Glucose; -- = Negative

Table 3 presents results of macroscopic and microscopic description of fungal isolates from the fruit samples used for the research. The profile of the postharvest fruit spoilage mycoflora isolated from watermelon (WL), pineapple (PA), and pawpaw (PP) is shown by the morphological data in the table. These fruits are extremely vulnerable to fungal colonization once their protective exocarp (peel) is broken because of their high water activity and high soluble sugar content. (Kwon-Ndung et al., 2022). Rhizopus was observed in (PP, WL, PA) | Mucor was observed in (WL, PA, PP). The observation of quick, "cotton wool-like" aerial mycelia and non-septate hyphae carrying sporangia provides morphological support that aligns with traditional descriptions of zygomycetes. Mucor has erect, branched sporangiophores and lacks rhizoids, which are root-like structures that anchor the sub-globose sporangiophores in Rhizopus.

Table 3: Macroscopic and Microscopic Description of Fungal Isolates

Fruits organisms were isolated from	**Organism**	**Macroscopic**	**Microscopic**
Watermelon Pineapple Pawpaw	*Mucor* sp.	Cotton wool like, aerial mycelia, first its grey white then it becomes darker in the colony.	Non-septate mycelium that bears sporangia scattered over the mycelia. Sporangia are erect and branched
Watermelon Pawpaw	*Aspergillus niger*	Dark brown colony with dense growth that grows to cover plate.	Conidia, head are radiate, conidiophores is unbranded to rhizoid. Hyphae are septate.
Pawpaw Pineapple	*Fusarium* sp	White colony with mass rapid growth covering the surface.	Short crescent conidiophore microcodia hyphae septate.
Pawpaw, Pineapple Watermelon	*Saccharomyces* sp	White, circular, in small growing-like bacteria with palm wine smell	Oval shape
Pineapple Pawpaw	*Penicillium* sp	Green pigmentation, white background powdery surface, circular in shape with elevated centre	Conidiophore is septate, erect and branched
Pawpaw Watermelon Pineapple	*Rhizopus* sp	Crayish with dark brown spore and colony	Subglobase sporangiophores with rhizoid, non-septate.
Pineapple	*Candida* sp	Creamy, smooth, white to off-white colonies. Soft, pasty texture with smooth edges. Yeast-like odor.	Oval, budding yeast cells. Pseudohyphae and true hyphae present.

Rhizopus stolonifer and Mucor species are often isolated from soft-skinned fruits like watermelon and pawpaw in studies and postharvest surveys conducted throughout Nigeria (such as Utako fruit market surveys). Zygomycetes are opportunists because they lack cross-walls (non-septate hyphae), and their cellular fluids

move quickly, enabling explosive growth that can fill a petri dish or engulf a piece of fruit within 48 hours (Mairami & Abdullahi, 2026). Extracellular pectinases are produced in enormous quantities by these fungi. The central lamella of host plant cells is quickly broken down by these enzymes, resulting in "soft rot"—the watery, structural collapse frequently observed in damaged watermelon and pawpaw. As observed by (Bano et al., 2023), For Rhizopus to start an infection, the fruit cuticle must be broken mechanically or punctured by insects. The main cause of these illnesses is rough treatment during postharvest transportation in local markets. Aspergillus niger is clearly confirmed by the dark brown/black dense colony with radiating heads and unbranched conidiophores in (WL, PP). The traditional "brush-like" structure of Penicillium, as observed in (PA, PP), is described by the green, powdery colony with septate, upright, and branched conidiophores. A study by (Zakaria, 2024) observed that because Aspergillus species are extremely tolerant of acidic pH conditions, they frequently have the highest frequency of occurrence among fruit-spoiling mycoflora, which is very relevant to acidic fruit matrices like pineapple. Ochratoxin A and malformins can be produced by wild strains of A. niger, despite their widespread usage in industrial biotechnology. Patulin, a mycotoxin frequently found in rotting fruit, can be produced by Penicillium species. There is a major risk to food safety because to the isolates found in pineapple, watermelon, and pawpaw. Customers run the danger of developing acute food poisoning or long-term mycotoxin exposure if these fruits are processed into fresh juices or fruit salads without carefully removing any bruised areas. The distinguishing characteristic of the genus Fusarium, as observed in PA and PP (Pineapple and Pawpaw), is the white, quickly expanding mass with septate hyphae and characteristic crescent-shaped (sickle-shaped) conidia (Zakaria, 2023), Fusarium species, including F. oxysporum and F. solani, are known for producing pineapple core rot and pawpaw stem-end rot. They are powerful phytopathogens that can infect fruit fields before harvest and stay dormant until the fruit ripens and its defences weaken (Badiwe et al., 2025). Fusarium infections damages the inside tissue of the fruit, rendering it utterly unmarketable and causing huge financial losses for smallholder farmers and traders. Members of this genus produce hazardous trichothecene mycotoxins (e.g., fumonisins, T-2 toxin). Due to their isolated presence, pineapple and pawpaw fruits that exhibit dry or white cottony rot must be thrown out (Li et al., 2025). These two genera are exactly matched by the white/cream, smooth, pasty colonies that microscopically appear as oval, budding yeast cells and have a unique "palm wine" or yeast-like odour. As seen in (PA, PP) and (PP, PA, WL), the presence of pseudohyphae—elongated, connected budding cells—distinguishes Candida from Saccharomyces.

While filamentous moulds predominate visible decay, Candida spp. (approximately 34%) and Saccharomyces spp. (approximately 18%) represent the highest population percentage of the fruit yeast microbiome during the late ripening stages, according to a recent metabarcoding and culture-based study of fruit microbiomes (Windholtz et al., 2021). Seldom is Saccharomyces the main pathogen. Rather, it moves into tissue that Mucor or Aspergillus has already degraded, acting as a secondary colonizer (Aires et al., 2025). It feeds on the exposed, sugary cell sap, fermenting the sugars into carbon dioxide and ethanol. While Saccharomyces is largely benign, Candida species can act as opportunistic human pathogens. Their presence on raw fruits highlights potential hygiene deficits during handling and emphasizes the need for thorough washing of fruits before raw consumption (de Melo Pereira et al., 2022). The frequency of distribution of fungal isolates across the fruit samples reveals critical patterns about how different fungi colonize specific hosts.

Table 4 presents frequency of fungal isolates detected from the fruit samples. The prevalence of Mucor, Rhizopus, and Saccharomyces throughout all three fruits confirms their status as ubiquitous cosmopolitan fungus. In a comparable postharvest examination of fruits in Nigerian markets, (Umar et al., 2023) found that Rhizopus stolonifer and Mucor species had the highest frequency of presence on high-moisture fruits. Aspergillus thrives well in the ambient temperatures of tropical marketplaces. Its absence in pineapple sample is an unusual observation, yet literature says it may infect all three.

Table 4: Frequency of Fungal Isolates

Organisms	**Samples Codes**			
	Watermelon	Pawpaw	Pineapple	**Frequency**
Mucor sp	+	+	+	3
Aspergillus sp	+	+	-	2
Fusarium sp	-	+	+	2
Saccharomyces sp	+	+	+	3
Penicillium sp	-	+	+	2
Rhizopus sp	+	+	+	3
Candida sp	-	-	+	1

Additionally, the highly rough, pocketed exterior (eyes) of pineapples readily retains moisture and forms micro-aerophilic zones where Candida can grow slowly without being rinsed away.

The fresh-squeezed juice industry faces a safety risk due to Candida's particular affinity for pineapple. Candida includes species that are opportunistic human pathogens, such as Candida albicans or Candida tropicalis. Because manual peeling often forces surface Candida cells straight into the juice matrix, pineapple cores need to be thoroughly cleaned or thermally treated.

The percentage occurrence of frequency of bacteria/isolate in different sample is found in Figure 2. The study reveals a total of seven (7) genera of bacterial isolate namely- Micrococcus, Staphylococcus, Clostridium, Bacillus, Salmonella, E. coli, Enterococcus and seven fungal genera: such as Mucor sp, Aspergillus niger, Fusarium sp, Rhizopus, Saccharomyces, Penicillium, Candida.

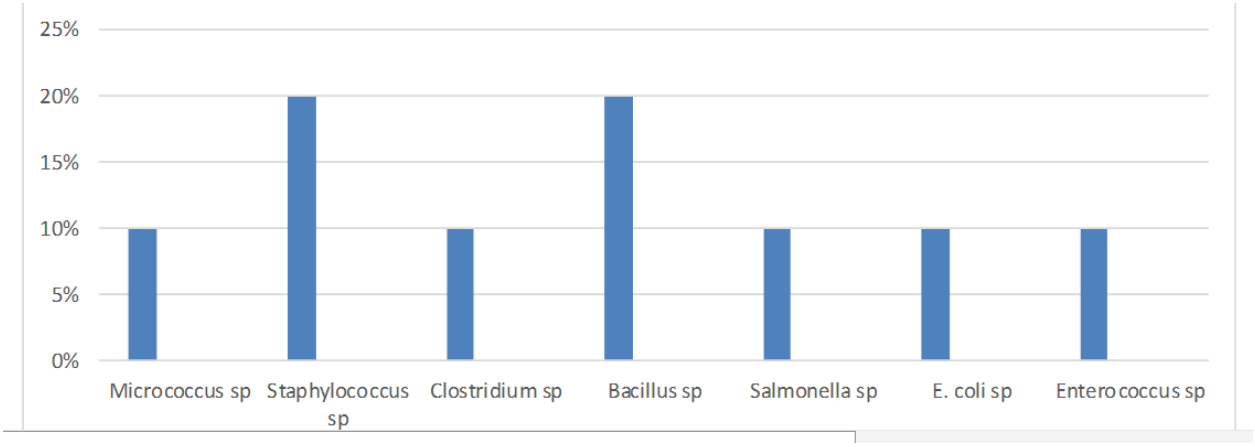


Figure 2: The percentage of bacteria that were isolated from the samples

The percentage occurrence profile of bacteria across samples showed that Acetobacter sp. had a list occurrence of 33.3%, Erwinia and Lactobacillus sp. had a percentage occurrence of 66.7%, and Staphylococcus aureus and Bacillus subtilis had a 100% occurrence.

The 100% incidence pattern of Bacillus subtilis and Staphylococcus aureus is highly consistent with current street-vended fruit surveys in Nigeria. (Olu-Taiwo et al., 2021) evaluated watermelon and ready-to-eat sliced pawpaw and found that Staphylococcus aureus frequently maintained incidence rates as high as 60% to 70% in raw sub-samples. Furthermore, market sanitation studies by (Biermann & Beutel, 2023) established that Bacillus species are universally isolated from retail produce because their endospores tolerate dry dust and environmental stress.

All three fruits have S. aureus, which is a clear indication of anthropogenic (human) contamination. S. aureus is a common commensal of human nasal passages and skin. Its 100% incidence suggests that merchants used inadequate personal hygiene when peeling, slicing, and packaging. Standard ambient cross-contamination from soil, dust, and unclean wooden cutting boards is reflected in the contemporaneous 100% baseline of B. subtilis.

Erwinia spp. and Lactobacillus spp. showed a 66.7% macro-occurrence, successfully growing colonies on pineapple and pawpaw samples but not registering on watermelon samples.

Erwinia species actively target highly textured composites like pineapple and fruits with thin or structurally sensitive skin like pawpaw (Tao et al., 2023). On the other hand, unless the fruit is severely crushed, good watermelon rinds provide a strong mechanical barrier that prevents Erwinia (Ben Romdhane et al., 2024). For Lactobacillus spp., (Ngouénam et al., 2021) found that the highly expressive, wet sugar sap of bruised pawpaw and cut pineapple core matrices is ideal for the growth of lactic acid bacteria.

Erwinia is an aggressive main tissue liquefier that breaks down plant cell walls with specific enzymes. Lactobacillus can enter the fruit because of this enzymatic breakdown, which leaves the inner fruit tissue deteriorated and watery. Long before pawpaws and pineapples reach the end of their natural shelf life,

Lactobacillus ferments the released plant sugars into organic acids, resulting in the strong, sour off-flavors that impair their edible quality (Tao et al., 2023).

The baseline boundary is marked by Acetobacter spp., which only occurred in pineapple (PA) and have a very limited macro-occurrence of 33.3%. is a specialized, acid-tolerant genus, according to ecological surveys of specialized food-borne bacteria (Olojede et al., 2025). Fruit sugars are quickly fermented into ethyl alcohol by Saccharomyces and Candida yeasts because pineapples are inherently acidic. By using its metabolic machinery to oxidize the ethanol straight into acetic acid, Acetobacter takes advantage of this niche. Pineapple juice and fresh chunks need special cold storage to prevent this particular pathway because it results in a strong, vinegar-like souring that drastically changes the fruit's odour character (Mota & Vilela, 2024). A serious public health warning is the 100% prevalence of Staphylococcus aureus. When permitted to grow at ambient tropical temperatures (over 25°C) on nutrient-rich substrates like cut fruit, some strains of S. aureus develop heat-stable enterotoxins. Customers are directly at risk of developing acute staphylococcal food poisoning if these sliced watermelons, pawpaws, or pineapples are kept out of the refrigerator on warm retail stands.

The experimental data in Table 5 evaluates the antibacterial activity of three native spices—Clove, Negro Pepper, and Aidan Fruit—extracted in 20 mL of ethanol against Staphylococcus sp., Bacillus sp., and Salmonella sp. Overall, the results demonstrate distinct variations in potency among the spices, as well as a clear dose-dependent response where increasing the weight of the spice leads to greater antibacterial efficacy.

Clove emerged as the most potent antibacterial agent among the three tested spices. Even at its lowest concentration of 2 g, Clove extract produced substantial zones of inhibition, reaching 44 mm for Staphylococcus sp., 16 mm for Bacillus sp., and 21 mm for Salmonella sp. All three bacterial strains were classified as completely susceptible (S) across all tested weights (2 g, 4 g, and 6 g). Maximum inhibition occurred at 6 g for Salmonella sp. (27 mm) and Bacillus sp. (19 mm), while Staphylococcus sp. maintained a consistently high inhibition zone of 44 mm across all concentrations.

Negro Pepper displayed moderate, concentration-dependent antibacterial activity. At the lowest weight of 8 g, the extract was insufficient to inhibit bacterial growth effectively, resulting in small zones of inhibition (6 mm to 13 mm) where all three bacterial species showed resistance (R). However, increasing the spice weight to 16 g and 24 g successfully triggered bacterial susceptibility (S). At 24 g, the zones of inhibition expanded to 18 mm for Staphylococcus sp., 20 mm for Bacillus sp., and 18 mm for Salmonella sp.

Aidan Fruit exhibited the weakest antibacterial properties. Across all tested concentrations (5 g, 10 g, and 15 g), the zones of inhibition remained minimal, ranging from 4 mm to 9 mm across all organisms. Consequently, Staphylococcus sp., Bacillus sp., and Salmonella sp. remained entirely resistant (R) to Aidan Fruit extract at every tested level, despite increases in weight.

Table 5: Susceptibility of the Pathogenic Organisms (Bacteria) to the Investigated Native Spices - Zone of Inhibition in mm

Organisms	**Clove**			**Negro Pepper**			**Aidan Fruit**		
Weight of Spices	2g	4g	6g	8g	16g	24g	5g	10g	15g
Ethanol (ml)	20	20	20	20	20	20	20	20	20
Zone of Inhibition (mm) (*Staphylococcus sp.*)	44	44	44	6	10	18	4	5	9
Degree of Susceptibility	S	S	S	R	S	S	R	R	R
Zone of Inhibition (mm) (*Bacillus* sp.)	16	18	19	8	14	20	4	5	7
Degree of Susceptibility	S	S	S	R	S	S	R	R	R
Zone of Inhibition (mm) (*Salmonella* sp.)	21	23	27	13	17	18	6	7	8
Degree of Susceptibility	S	S	S	R	S	S	R	R	R

Key: S – Sensitive to the test organism; R – Resistant to the test organism

Three local West African plant extracts—Clove (Syzygium aromaticum), Negro Pepper (Xylopia aethiopica),

and Aidan Fruit (Tetrapleura tetraptera) have been evaluated as natural antimicrobials against common food-borne and environmental bacteria (Staphylococcus sp., Bacillus sp., and Salmonella sp.) according to the susceptibility matrix.

All extracts exhibit a dose-dependent response, according to the data, with clove appearing as a particularly strong inhibitor, Negro Pepper exhibiting moderate activity at higher concentrations, and Aidan Fruit exhibiting weak antimicrobial performance within the studied parameters.

The antibacterial profile of clove was particularly active. It achieves a huge 44mm zone of inhibition against Staphylococcus sp., 16mm against Bacillus sp., and 21mm against Salmonella sp. even at its lowest tested weight (2g in 20ml ethanol), with slight incremental increases at 4g and 6g. Ethnobotanical literature validates this severe sensitivity profile (Bencheikh et al., 2024) and studies by (Ghoshal et al., 2025) noted that volatile phenolic molecule eugenol is found in remarkably high amounts in clove essential oils and extracts, eugenol is a well-known membrane disruptor that denatures important structural proteins, changes the permeability of bacterial cells, and causes intracellular leakage. Clove's potential as a highly effective biopreservative for sliced fruits is highlighted by the fact that Staphylococcus sp. is incredibly sensitive (44mm) to even the lowest 2g concentration. Using moderate, food-grade clove compositions could totally eliminate surface contamination because Staphylococcus was detected from 100% of fruit samples as a result of human handling.

There was a clear threshold effect in Negro Pepper. All three target bacterial taxa are categorised as Resistant (R) at the lowest dose (8g), with tiny zones measuring 6–13 mm. But a shift to Sensitive (S) is triggered by doubling and tripling the weight to 16g and 24g, reaching a maximum of 20mm for Bacillus sp. and 18mm for Staphylococcus and Salmonella (Adegbenu et al., 2020). Research by Fleischer et al 2008 on Nigerian medicinal plants demonstrated that Xylopia aethiopica had mild to potent antibacterial action, which is fuelled by bioactive diterpenes, kauranes, and essential oils like linalool and xylopic acid. However, these substances frequently need a higher concentration threshold to effectively penetrate the outer lipopolysaccharide membrane of Gram-negative pathogens (Salmonella) or the thick peptidoglycan walls of Gram-positive organisms (Bacillus, Staphylococcus). Negro pepper is a good biocontrol substitute, however in order to ensure food safety, it must be used at larger structural payloads (at least 16g per 20ml solvent pool). Its application would be more appropriate for savoury or specialised spiced fruit processing than for fresh, sweet fruit slices because of its high concentration dependency, which may create strong, peppery flavour characteristics.

Aidan Fruit was unable to control any of the test pathogens at any of the three weights (5g, 10g, and 15g). The zones of inhibition, which ranged from a low of 4 mm to a maximum of 9 mm, remained insignificant, leading to a consistent resistant classification throughout. Tetrapleura tetraptera is abundant in flavonoids, tannins, and saponins, which frequently have positive effects on general health (Mensah et al., 2024).

According to (Afrendi et al., 2023) phytotherapy studies, the direct bactericidal action of raw or ethanol-extracted Aidan fruit pulp is often significantly lower than that of spice seeds or flower buds such as cloves. Bacterial proliferation is frequently not stopped by the amount of active triterpenoid saponins in a straightforward crude ethanol wash.

Although Aidan fruit is widely used in traditional medicine and local soups, it cannot be relied upon as a primary postharvest sanitiser or biopreservative against aggressive bacterial contaminants on fruits. Both Bacillus and Staphylococcus are Gram-positive, but Bacillus sp. exhibits greater structural resistance (16mm to 19mm) under Clove extract. This resilience is probably due to the physiological toughness of Bacillus species, which are classic endospore-formers that can produce extremely protective cell layers (Mohamadzadeh & Abbaspour, 2024). Many antimicrobials originating from plants are typically blocked by the complex outer membrane of Salmonella sp., a gram-negative bacterium. It's interesting to note that the research data indicates that Salmonella sp. reacts well to greater dosages of Negro Pepper (18 mm) and is extremely sensitive to Clove (21 mm to 27 mm). This suggests that clove and black pepper's active volatiles are very good at rupturing outer lipid envelopes.

Table 6 reveals the antifungal effectiveness of three native African spices extracted with ethanol: Aidan Fruit (Tetrapleura tetraptera), Negro Pepper (Xylopia aethiopica), and Clove (Syzygium aromaticum) against three different fungal groups: Aspergillus niger (filamentous mould), Penicillium (mould), and Saccharomyces (yeast). All examined fungal strains demonstrated absolute susceptibility (S) across all weight parameters,

which is a crucial finding throughout the entire dataset and confirms that these native spices contain strong, wide-spectrum bioactive antifungal chemicals. In this investigation, clove showed the most impressive and potent antifungal profile, especially at lower doses. Clove's maximum zone of inhibition against Penicillium was 44 mm, and it was entirely constant at all tested weights. This shows that a maximal saturation of fungal growth inhibition was already reached at the lowest threshold examined. Regarding Saccharomyces: As the weight of the spice rose, the zone gradually widened, going from 30 mm to 33 mm and reaching a high at 36 mm. Clove demonstrated a dramatic, concentration-dependent rise from 13 mm to 38 mm for Aspergillus niger.

Table 6: Susceptibility of the Pathogenic Organisms (Fungi) to the Investigated Native Spices - Zone of Inhibition in mm

Organisms	**Clove**			**Negro Pepper**			**Aidan Fruit**		
Weight of Spices (g)	2	4	6	8	16	24	5	10	15
Ethanol (ml)	20	20	20	20	20	20	20	20	20
Zone of Inhibition (mm) (*Saccharomyces*)	30	33	36	11	13	20	17	28	30
Degree of Susceptibility	S	S	S	S	S	S	S	S	S
Zone of Inhibition (mm) (*Penicillium*)	44	44	44	33	27	28	21	22	27
Degree of Susceptibility	S	S	S	S	S	S	S	S	S
Zone of Inhibition (mm) (*Aspergillus niger*)	13	16	38	10	12	14	7	9	11
Degree of Susceptibility	S	S	S	S	S	S	S	S	S

Key: S – Sensitive to the test organism; R – Resistant to the test organism

Mycological literature provides ample evidence of clove's unmatched potency. Eugenol (4-allyl-2-methoxyphenol), a volatile phenolic molecule that readily penetrates fungal cell membranes, is abundant in clove extract, according to earlier research (Liñán-Atero et al., 2024). A study by (Pandey et al., 2024) on botanicals against Aspergillus niger confirmed that clove extracts completely suppressed mycelial growth compared to other local spices. Similarly, findings by (Bai et al., 2023) showed that the high phenolic profile of cloves disrupts the cell wall envelope, causing cellular leakage in resistant moulds such as Aspergillus and Penicillium species.

One particularly effective bio-fungicide is clove. It is a very economical choice for pharmaceutical applications and natural food preservation because it reaches huge inhibitory brackets at very low material weights, reducing the real amount of raw spice required to eradicate fungal diseases. Clove and Negro spices produced different levels of fungal activity against every fungal bio-test, which is consistent with the results of (Tshabalala et al., 2021) in there study on effect of clove (Syzygium aromaticum) spice as microbial inhibitor of resistant bacteria and on organoleptic quality of meat. For two of the target organisms, Negro Pepper showed a clear, consistent, concentration-dependent trend in which an increase in spice weight directly correlated with higher antifungal activity. The inhibitory zone grew linearly with concentration against Saccharomyces, going from 11 mm to 13 mm and then to 20 mm. In opposition to Aspergillus niger, it increased gradually from 10 mm to 14 mm. Against Penicillium: It's interesting to note that Negro Pepper defied expectations in this instance. After reaching its lowest weight of 33 mm, it fell to 27 and 28 mm. Bioactive antagonism, also known as saturation-precipitated agglomeration, is a known phenomenon that explains the unusual reaction observed in Penicillium, where higher extract weights produced somewhat lower inhibitory zones. Fruit oils from Xylopia aethiopica have been shown to contain high levels of 1,8-cineole and diterpenes, such as kaurane derivatives. These complex phytochemicals may combine or compete for binding sites in crude ethanolic extractions if the weight threshold is exceeded. This can occasionally lower the clean diffusion rate of the active substances through the agar matrix. However, its strong baseline activity is in complete agreement with research by (G et al., 2024), who effectively suppressed food-spoilage yeasts such Saccharomyces cerevisiae using ethanolic extracts of Xylopia aethiopica, solidifying its worth as a food protectant.

Negro pepper works wonders, but it needs to be applied carefully. Using a lower, optimised dose is actually

more harmful to the fungus than over-concentrating the formula for some moulds, such as Penicillium. Activity increased from 17 to 30 mm as a result of Aidan Fruit's action against Saccharomyces. It clearly climbed up against Penicillium, going from 21 mm to 27 mm. Aidan fruit displayed a small but consistent clean zone against Aspergillus niger, growing from 7 mm to 11 mm. The clean, linear extraction of Aidan Fruit's primary secondary metabolites is indicated by its consistent, predictable climbing efficacy. Oleanolic acid glycosides (saponins) and tannins are abundant in Tetrapleura tetraptera, according to phytochemical evaluations. The sterols (ergosterol) imbedded in fungal plasma membranes are immediately bound by saponins, which function as natural detergents and cause cell lysis (rupture). The zones of inhibition extended so smoothly with the weight increases in this data because alcoholic extractions easily extract these polar saponins, as demonstrated by historical West African pharmacological surveys. Aidan Fruit is a very dependable and consistent botanical choice. It is a great option for industrial food preservation, standardized antimicrobial washes, and holistic skin treatments where dose-tailored control is required because its zone of inhibition consistently increases with dosage. Although Aspergillus niger was the most resilient organism overall (producing generally smaller zones of inhibition across all three spices), it was nevertheless successfully repelled, according to the overall susceptibility patterns, which show a clear hierarchy among the spices. In the end, the information supports the customary use of these indigenous spices in ethnomedicine and highlights their enormous potential as organic, clean substitutes for artificial chemical preservatives.

Conclusion

The result obtained showed the presence of microbes that contaminate fruits that are ready to eat bought at three Port Harcourt markets, probably due to poor sanitary practices. For example, peeling the fruit with ordinary hand, with contaminated hand that is not washed, hands contaminated with public food after eating and in some cases, skin surface with rash and other fungal – oriented skin infection.

The findings of this study provide clear evidence that clove (Syzygium aromaticum) possesses a remarkably higher antimicrobial efficacy compared to Negro pepper (Xylopia aethiopica) and Aidan fruit (Tetrapleura tetraptera). The ethanol extracts of clove demonstrated significant inhibitory effects against all six test organisms—three fungal isolates (Saccharomyces, Penicillium, and Aspergillus) and three bacterial isolates (Salmonella, Bacillus, and Staphylococcus). This consistent broad-spectrum activity highlights clove's superior potential as a natural antimicrobial agent. In contrast, Negro pepper exhibited only moderate inhibitory activity, suggesting that although it contains bioactive compounds, its concentration or potency may not be as strong as that of clove under the conditions tested. Aidan fruit recorded the least antimicrobial activity, which indicates a lower presence or weaker expression of antimicrobial phytochemicals in its extract. Nevertheless, the ability of both Negro pepper and Aidan fruit to inhibit growth, even at a lesser degree, confirms that they still possess valuable antimicrobial properties that should not be disregarded. Results of this study therefore reinforces the traditional use of clove in food preservation and medicine, while scientifically validating its role as an effective natural antimicrobial agent. The findings have important implications for food safety, particularly in the preservation of ready-to-eat fruits that are highly prone to microbial contamination. By demonstrating that clove extracts can inhibit both bacterial and fungal growth. This research supports the possibility of reducing reliance on synthetic chemical preservatives, which are often associated with health risks and environmental concerns.

This study concludes that clove is the most effective of the three spices tested, with strong potential for application in antimicrobial treatments and natural food preservation systems. Its use can enhance public health safety by reducing the risks of foodborne infections, prolonging the shelf-life of perishable fruits, and offering an eco-friendly alternative to chemical preservatives. This work also opens avenues for further research into the development of clove-based antimicrobial formulations, as well as the exploration of synergistic effects when combined with other plant-derived products. The spices could be used for the treatment of individual that may be affected through the consumption of the ready-to-eat fruit. Precautionary measure should be taken when displaying peeled ready-to-eat fruit to prevent contamination and aerosol microbes on the food items.

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