ANTIMICROBIAL ACTIVITY OF *XYLOPIA AETHIOPICA* EXTRACT ON MICROORGANISMS ASSOCIATED WITH THE THE SPOILAGE OF VEGETABLES IN MILE 3 MARKET, PORTHARCOURT.

ABSTRACT

With increasing demand for natural preservatives, this study explores the effectiveness of *Xylopia aethiopica* extracts in reducing vegetable spoilage. The aim was to evaluate these extracts' potential in inhibiting spoilage microorganisms, determine the most effective concentration for preservation, and assess their impact on sensory qualities. Conducted in Mile 3 Market, Port Harcourt, Nigeria, vegetables (onions, tomatoes, and carrots) were purchased from vendors and transported to a microbiological laboratory for analysis. Vegetable samples were treated with ethanol and aqueous extracts of *Xylopia aethiopica* at concentrations of 10%, 20%, and 30% (w/v). Microbial and fungal counts were measured over 14 days. Results showed that the 30% ethanol extract was the most effective, significantly reducing microbial and fungal growth compared to the control and other treatments. Key microorganisms isolated included *Bacillus cereus, Pseudomonas aeruginosa, Aspergillus niger* and *Penicillium spp*. ANOVA analysis confirmed significant reductions in microbial counts in treated vegetables ($p < 0.05$).Sensory evaluation revealed that the extracts, particularly the 30% ethanol extract, did not negatively affect the vegetables' taste, smell, or appearance. Phytochemical analysis indicated that the ethanol extract had higher concentrations of antimicrobial bioactive compounds. This study demonstrates the potential of *Xylopia aethiopica* as an effective natural preservative, offering a promising alternative to synthetic preservatives. Future research should focus on long-term effects, broader applications, and consumer acceptance for practical implementation.

KEYWORDS: *Xylopia aethiopica*, phytochemicals, Anova

INTRODUCTION

Vegetable spoilage is a significant issue in food security, particularly in developing countries where preservation methods are often limited. Microbial contamination is one of the leading causes of vegetable spoilage, contributing to substantial post-harvest losses. In Mile 3 Market, a major trading hub in Port Harcourt, Nigeria, the problem of vegetable spoilage is exacerbated by poor hygiene practices, inadequate storage facilities, and the humid tropical climate, which creates a conducive environment for the growth of spoilage microorganisms. Understanding and controlling these microorganisms is essential to reduce spoilage and extend the shelf life of vegetables, which are a staple in the diet of many Nigerians (Karanth et al., 2023).

Xylopia aethiopica, commonly known as "African pepper" or "Negro pepper," is a plant native to West Africa and widely used in traditional medicine and cuisine. The dried fruits of *Xylopia aethiopica* have been reported to possess various pharmacological properties, including antimicrobial, antioxidant, and anti-inflammatory activities . The plant's antimicrobial properties have garnered significant attention, particularly in the context of traditional medicine, where it is used to treat various infections and diseases (Karanth et al., 2023) .

The antimicrobial activity of *Xylopia aethiopica* is attributed to its rich phytochemical composition. Studies have identified a range of bioactive compounds in the plant, including alkaloids, tannins, flavonoids, and essential oils, which have demonstrated efficacy against a wide spectrum of microorganisms (Fleischer et al., 2008) . These properties make *Xylopia aethiopica* a promising candidate for developing natural preservatives, especially in regions like Mile 3 Market, where the high cost and limited availability of synthetic preservatives pose a challenge.

Microorganisms associated with vegetable spoilage, such as *Escherichia coli, Salmonella spp., Staphylococcus aureus,* and various molds and yeasts, are of particular concern in the Mile 3 Market. These microorganisms not only cause rapid deterioration of vegetables but also pose significant health risks to consumers (Degaga et al., 2022). The conventional approach to managing vegetable spoilage in such settings involves improving storage conditions and using chemical preservatives. However, chemical preservatives often raise concerns regarding safety, environmental impact, and the development of resistant strains of microorganisms (Degaga et al., 2022).

Given the challenges associated with synthetic preservatives, there is a growing interest in exploring natural alternatives that are both effective and safe. *Xylopia aethiopica* offers a potential solution, as its antimicrobial properties could be harnessed to inhibit the growth of spoilage microorganisms and thereby extend the shelf life of vegetables. Research in this area is crucial to validate the efficacy of *Xylopia aethiopica* against specific spoilage microorganisms and to determine the practical applications of this plant in food preservation (Degaga et al., 2022).

The challenges posed by microbial spoilage of vegetables are not only a concern for food safety but also for the economic well-being of market traders and consumers. In Mile 3 Market, vegetables are sold in large quantities daily, with much of the produce coming from surrounding rural areas. However, due to the high ambient temperatures and humidity, vegetables are highly susceptible to microbial invasion, leading to spoilage before they can be sold or consumed. The microorganisms responsible for this spoilage are diverse, including bacteria, yeasts, and molds, each of which can cause different forms of deterioration, such as soft rot, discoloration, and off-odors .

The economic losses incurred from vegetable spoilage are significant, especially in markets like Mile 3, where many traders rely on daily sales for their livelihood (Balali et al., 2020). The rapid spoilage also leads to price fluctuations and food shortages, further exacerbating food insecurity in the region. Moreover, the consumption of spoiled vegetables can lead to foodborne illnesses, which are a major public health concern in Nigeria. The need for effective, affordable, and locally available solutions to prevent vegetable spoilage is therefore critical.

*Xylopia aethiopic*a has long been used in traditional African medicine to treat a variety of ailments, including infections caused by bacteria and fungi. Its use as a spice in cooking also hints at its potential role as a natural preservative, as many culinary spices have been historically used to extend the shelf life of food. The phytochemicals present in *Xylopia aethiopica*, such as diterpenes, xylopic acid, and sesquiterpenes, have shown significant antimicrobial activity in various studies . These compounds work by disrupting the cell membranes of microorganisms, inhibiting their growth, and ultimately leading to cell death. Given its antimicrobial properties, *Xylopia aethiopica* could serve as an effective natural preservative for vegetables, particularly in traditional markets where refrigeration is limited or nonexistent. Studies have shown that extracts from *Xylopia aethiopica* can inhibit the growth of a variety of foodborne pathogens, including *Escherichia coli, Staphylococcus aureus,* and *Salmonella spp*., all of which are commonly associated with food spoilage and foodborne diseases (Juliani et al., 2008).

These findings suggest that incorporating *Xylopia aethiopica* into preservation strategies could help reduce spoilage rates, thereby improving the quality and safety of vegetables sold in Mile 3 Market. The use of natural preservatives like *Xylopia aethiopica* aligns with the global trend towards reducing the reliance on synthetic chemicals in food production. Consumers are increasingly concerned about the potential health risks associated with synthetic preservatives, which have been linked to various adverse effects, including allergic reactions and the development of antibiotic-resistant bacteria. By contrast, natural preservatives are generally perceived as safer and more environmentally friendly, making them more acceptable to consumers. However, while the antimicrobial properties of *Xylopia aethiopica* are well-documented, it's practical application in preserving vegetables remains underexplored. There is a need for detailed studies that not only confirm the efficacy of *Xylopia aethiopica* against spoilage microorganisms in real-world conditions but also determine the optimal methods of application, such as concentration, form (e.g., powder, extract, or essential oil), and method of incorporation into storage practices. It is also important to evaluate the potential sensory impacts of using *Xylopia aethiopica* as a preservative (Bagwai et al., 2020). While its strong aromatic properties may be desirable in certain contexts, they could alter the taste or smell of vegetables, potentially affecting consumer acceptance. Therefore, research into the organoleptic properties of *Xylopia aethiopica*-treated vegetables is essential to ensure that the benefits of reduced spoilage do not come at the cost of consumer satisfaction. Studies have shown that *Xylopia spp*., a plant species native to West Africa, possesses potent antimicrobial properties . This presents an opportunity to investigate its potential use as a natural preservative for vegetables commonly sold at local markets like Mile 3 Market. Given the limited research on *Xylopia* extract's efficacy against vegetable spoilage microorganisms in a real-world market setting, this study seeks to address this gap and explore its practical application within the context of local agricultural practices.

Xylopia aethiopica, commonly known as African pepper, belongs to the *Annonaceae* family and the *Xylopia* genus. It is a tropical plant known for its aromatic seeds and fruit, which are frequently used in both cooking and traditional medicine (Hutchinson & Dalziel, 1954). The

seeds are found inside a woody, aromatic fruit that splits open when ripe. This fruit and its seeds are celebrated for their antimicrobial properties, making them valuable in culinary and medicinal contexts .

The plant's composition includes essential oils like xylopic acid, alkaloids, flavonoids, and phenolic compounds. These compounds contribute to its antimicrobial, antioxidant, and anti-inflammatory effects . *Xylopia aethiopica* is particularly noted for its ability to combat a wide range of microorganisms, which makes it a promising natural preservative (Bagwai et al., 2020).

Vegetables, rich in fiber, vitamins, and antioxidants, are essential for human health. However, they are prone to microbial spoilage, which affects their quality and shelf life. Common preservation methods such as refrigeration, canning, and drying help extend the (Petcu et al., 2023) shelf life of vegetables, but they may alter the taste and texture of the produce (Mungofa et al., 2022).

The antimicrobial properties of *Xylopia aethiopica* are particularly relevant in vegetable preservation. Its essential oils exhibit broad-spectrum antimicrobial activity, making it an effective alternative to synthetic preservatives (Fleischer et al., 2008) . When used to protect vegetables from microbial contamination, *Xylopia aethiopica* can help extend their shelf life without compromising their quality, though careful consideration of its impact on taste and aroma is necessary for consumer acceptance.

METHODOLOGY

The study was conducted at Mile 3 Market in Port Harcourt, Rivers State, Nigeria, focusing on fresh vegetables like onions, tomatoes, and carrots, known for their susceptibility to spoilage. Vegetables were collected from different vendors, selected based on freshness and uniformity, and transported to the laboratory in sterile bags. They were washed with sterile water and air-dried.

Xylopia aethiopica fruits were sourced from a local herbal market, authenticated, cleaned, and air-dried. The dried fruits were ground into powder and extracted with ethanol and water. The ethanol extract was obtained by soaking the powdered fruit in 95% ethanol for 48 hours, filtered, and concentrated. The aqueous extract was similarly prepared using distilled water and concentrated.

Phytochemical analysis was conducted on both extracts to identify active compounds like alkaloids, flavonoids, saponins, tannins, phenolics, and glycosides. The ethanol extract showed higher concentrations of these compounds, indicating its greater potential as a natural preservative.

Vegetables were divided into groups and treated with different concentrations (10%, 20%, and 30%) of ethanol and aqueous extracts. A control group was treated with sterile water. After

immersion, the vegetables were air-dried. Microbial analysis was performed by swabbing the vegetable surfaces at different time intervals (days 0, 3, 7, and 14), followed by inoculation onto nutrient agar plates. The plates were incubated, and colony-forming units (CFUs) were counted.

Staining techniques, including Gram and lactophenol cotton blue stains, were used to identify bacterial and fungal isolates. Biochemical tests (e.g., catalase, oxidase, indole, citrate, and urease) were conducted to further characterize the bacteria.

Sensory evaluation was carried out to assess the taste, smell, and appearance of the treated vegetables, using a trained panel. Data were analyzed using descriptive statistics and ANOVA, with significance set at $p < 0.05$. The study provided a comprehensive evaluation of *Xylopia aethiopica* as a natural preservative for vegetables.

RESULT

Table 1 Shows that the total bacterial count on the vegetables increased over time in all groups. However, the treated vegetables (both ethanol and aqueous extracts) exhibited significantly lower bacterial counts compared to the control group. The 30% ethanol extract was the most effective, resulting in the lowest bacterial counts at all-time points. Statistical analysis (ANOVA) as seen in appendix 3 (anoval table 1) confirmed that there was a significant reduction in bacterial growth in the treated groups compared to the control ($p < 0.05$).

As seen in Table 2, the fungal count increased significantly in the control group, while the treated groups showed much lower fungal growth. The 30% ethanol extract again proved to be the most effective, with the lowest fungal counts at all-time points. ANOVA analysis showed a significant reduction in fungal growth in treated vegetables compared to the control ($p < 0.05$) as show in appendix 3 (anoval table 1).

Bacillus cereus was the most frequently isolated microorganism, with a high occurrence in tomatoes at 40%, followed by carrots at 35%, and onions at 30%. The overall occurrence across all vegetables was 35%.

In table 3, *Pseudomonas aeruginosa* had the highest occurrence in tomatoes at 50%, and was also commonly found in carrots at 30% and onions at 20%, leading to an overall occurrence of 33.3%. *Aspergillus niger* was more commonly isolated from onions with an occurrence of 25%, and had lower occurrences in carrots and tomatoes at 20% and 15%, respectively. The overall occurrence was 20%.

Penicillium spp. was evenly distributed across the vegetables, with the highest occurrence in carrots at 25%, followed by tomatoes at 20%, and onions at 15%. This resulted in an overall occurrence of 20%. *Escherichia coli* was most frequently found in tomatoes at 25%, with similar occurrences in carrots at 20% and onions at 10%, leading to an overall occurrence of 18.3%.

Saccharomyces cerevisiae had the lowest overall occurrence, being most present in carrots at 15%, followed by tomatoes at 10% and onions at 5%, with an overall occurrence of 10%.

The sensory evaluation (Table 4) indicated that the *Xylopia aethiopica* extracts did not negatively affect the taste, smell, or appearance of the treated vegetables. In fact, higher concentrations of the extracts, particularly the 30% ethanol extract, received higher sensory scores compared to the control. ANOVA analysis showed significant differences in sensory scores between treatments and the control ($p < 0.05$) as show in appendix 3 (anova table 2)

The phytochemical (table 5) analysis revealed that the ethanol extract of *Xylopia aethiopica* had higher concentrations of alkaloids, flavonoids, saponins, tannins, and phenolics compared to the aqueous extract. These bioactive compounds are known for their antimicrobial properties, which likely contributed to the effectiveness of the extracts in inhibiting spoilage microorganisms.

Table 1: Total Bacterial Count (CFU/mL) on Treated and Control Vegetables

Treatment	Day 0	Day 3	Day 7	Day 14
Control (Sterile Water)	0	1.5×10^4	4.5×10^5	9.2×10^5
10% Ethanol Extract	0	1.0×10^3	2.3×10^4	4.0×10^4
20% Ethanol Extract	0	5.0×10^2	8.0×10^3	2.2×10^4
30% Ethanol Extract	0	1.0×10^2	3.0×10^3	1.0×10^4
10% Aqueous Extract	0	2.0×10^3	3.0×10^4	6.5×10^4
20% Aqueous Extract	0	1.5×10^3	1.5×10^4	3.2×10^4
30% Aqueous Extract	0	5.0×10^2	5.0×10^3	1.2×10^4

Table 2: Total Fungal Count (CFU/mL) on Treated and Control Vegetables

Treatment	Day 0	Day 3	Day 7	Day 14
Control (Sterile Water)	0	1.0×10^3	2.5×10^4	5.8×10^4
10% Ethanol Extract	0	5.0×10^2	1.2×10^3	2.4×10^3
20% Ethanol Extract	0	2.5×10^2	8.0×10^2	1.5×10^3
30% Ethanol Extract	0	1.0×10^2	3.5×10^2	8.0×10^2
10% Aqueous Extract	0	7.5×10^2	1.5×10^3	3.0×10^3
20% Aqueous Extract	0	5.0×10^2	1.0×10^3	2.0×10^3
30% Aqueous Extract	0	2.0×10^2	5.5×10^2	1.2×10^3

Table 3: Percentage Occurrence of Isolated Microorganisms in Vegetables

Microorganism	Onions (%)	Tomatoes (%)	Carrots (%)	Overall (%)
Bacillus cereus	30	40	35	35
Pseudomonas aeruginosa	20	50	30	33.3
Aspergillus niger	25	15	20	20
Penicillium spp.	15	20	25	20
Escherichia coli	10	25	20	18.3
Saccharomyces cerevisiae	5	10	15	10

Table 4: Sensory Evaluation Scores (Mean ± SD)

Treatment	Taste (5-point scale)	Smell (5-point scale)	Appearance (5-point scale)
Control (Sterile Water)	3.5 ± 0.5	3.0 ± 0.4	2.8 ± 0.6
10% Ethanol Extract	4.2 ± 0.4	4.0 ± 0.3	4.0 ± 0.4
20% Ethanol Extract	4.5 ± 0.3	4.4 ± 0.3	4.5 ± 0.3
30% Ethanol Extract	4.7 ± 0.2	4.6 ± 0.2	4.8 ± 0.2
10% Aqueous Extract	4.0 ± 0.4	3.8 ± 0.4	3.9 ± 0.4
20% Aqueous Extract	4.3 ± 0.3	4.1 ± 0.3	4.2 ± 0.3
30% Aqueous Extract	4.6 ± 0.2	4.4 ± 0.3	4.6 ± 0.2

Table 3: Phytochemical Composition of *Xylopia aethiopica* Extracts

Phytochemical	Ethanol Extract (%)	Aqueous Extract (%)
Alkaloids	2.5	1.8
Flavonoids	3.1	2.5
Saponins	1.4	0.9
Tannins	2.2	1.6
Phenolics	4.3	3.7
Glycosides	1.1	0.8

DISCUSSION AND CONCLUSION

The findings from this study underscore the significant effectiveness of *Xylopia aethiopica* extracts, particularly the 30% ethanol extract, in inhibiting the growth of spoilage microorganisms on vegetables. These results align well with previous research, which highlighted the potent antimicrobial properties of *Xylopia aethiopica* due to its rich phytochemical composition (Japhet et al., 2024).

The study demonstrated a noticeable reduction in both bacterial and fungal counts in the vegetables treated with *Xylopia aethiopica* extracts compared to the untreated control group. Over time, the bacterial and fungal counts in the control group increased significantly, which is consistent with the natural progression of spoilage in fresh produce. This spoilage is often exacerbated by the presence of spoilage microorganisms such as *Bacillus cereus* and *Pseudomonas aeruginosa* for bacteria, and *Aspergillus niger* and *Penicillium spp.* for fungi, as evidenced by the data presented in the tables. The increase in microbial counts in the untreated vegetables is a common occurrence documented in numerous studies, reflecting the high susceptibility of fresh vegetables to microbial contamination, which can lead to rapid spoilage and potential health risks (Sokamte et al., 2018).

The significant reduction in microbial counts observed in vegetables treated with *Xylopia aethiopica* extracts can be attributed to the presence of bioactive compounds such as alkaloids, flavonoids, saponins, tannins, and phenolics. These compounds were found in higher concentrations in the ethanol extract, as highlighted in the phytochemical analysis. Such bioactive compounds are well-known for their antimicrobial properties, which include disrupting microbial cell membranes and inhibiting the growth and proliferation of pathogens. This aligns with previous research findings where ethanol extracts of various plants exhibited stronger antimicrobial activity compared to aqueous extracts, likely due to the ability of ethanol to extract a higher concentration of these potent phytochemicals (Dzoyem et al., 2017) .

The high occurrence of *Bacillus cereus* in tomatoes and carrots, with frequencies of 40% and 35% respectively, highlights the vulnerability of these vegetables to this particular bacterium. *Bacillus cereus* is notorious for causing foodborne illnesses through the production of toxins, and its high prevalence in commonly consumed vegetables raises significant food safety concerns. Similarly, the presence of *Pseudomonas aeruginosa* in high proportions, particularly in tomatoes, is concerning due to this bacterium's association with spoilage. *Pseudomonas aeruginosa* is known for producing a biofilm, which not only contributes to spoilage but also makes the bacterium more resistant to conventional preservation methods (Yu et al., 2020). The detection of *Aspergillus niger* and *Penicillium spp.* primarily in onions and carrots is equally noteworthy, as these fungi are responsible not only for spoilage but also for the production of mycotoxins. Mycotoxins pose serious health risks, including carcinogenic effects, making the inhibition of these fungi in food products critical for public health .

The sensory evaluation results suggest that the use of *Xylopia aethiopica* extracts, particularly at higher concentrations such as the 30% ethanol extract, does not negatively affect the taste, smell, or appearance of the treated vegetables. This finding is significant as it addresses one of the primary challenges in using natural preservatives—maintaining the sensory qualities of food products (Japhet et al., 2024). Notably, the higher sensory scores for vegetables treated with the 30% ethanol extract indicate that not only is the extract effective in microbial inhibition, but it may also enhance the sensory appeal of the vegetables. This enhancement is likely due to the prevention of off-flavors and odors that are typically associated with microbial spoilag.

The practical implications of these findings are profound, particularly for the use of *Xylopia aethiopica* extracts in food preservation. The study's results suggest that these extracts can be effectively applied in market settings where fresh vegetables are sold, which are often prone to rapid spoilage. Implementing methods such as direct immersion in the extract, which was shown to be effective in this study, could be a simple yet highly effective strategy to extend the shelf life of fresh produce. This not only reduces food waste but also helps to mitigate economic losses for vendors, who often struggle with the rapid spoilage of their goods (Etebu & Enaregha, 2013).

From a health perspective, the ability of *Xylopia aethiopica* extracts to inhibit spoilage microorganisms has significant implications. By reducing the levels of spoilage microorganisms like *Bacillus cereus* and *Escherichia coli*, which were frequently isolated in this study, the risk of foodborne illnesses associated with these pathogens is substantially lowered. This is particularly important given the widespread consumption of fresh vegetables and the potential health risks posed by contaminated produce (Abarikwu et al., 2016). Furthermore, the inhibition of fungi like *Aspergillus niger* reduces the risk of mycotoxin exposure, which is a serious public health concern due to the carcinogenic potential of these compounds. The findings of this study support the potential of *Xylopia aethiopica* extracts as a natural and effective means of preserving fresh vegetables. The extracts not only inhibit spoilage microorganisms but also maintain or even enhance the sensory qualities of the vegetables. This aligns with previous research on the antimicrobial efficacy of plant extracts and highlights the importance of phytochemicals in developing food preservation strategies that are both effective and safe for consumers.

CONCLUSION

The results of this study provide compelling evidence that *Xylopia aethiopica* extracts, particularly the 30% ethanol extract, are highly effective in reducing microbial growth and preserving the quality of fresh vegetables. This conclusion is drawn from the significant reduction in both bacterial and fungal counts observed in treated vegetables compared to the control group, with the 30% ethanol extract consistently showing the greatest efficacy. The phytochemical analysis further supports these findings, revealing that the ethanol extract of *Xylopia aethiopica* contains higher concentrations of bioactive compounds known for their antimicrobial properties, such as alkaloids, flavonoids, saponins, tannins, and phenolics (Abarikwu et al., 2016) .

These findings align with previous research that has demonstrated the potent antimicrobial activity of *Xylopia aethiopica* due to its rich phytochemical profile (Ejelonu et al., 2011; Ogbonna et al., 2013). The ability of these extracts to significantly inhibit the growth of common spoilage microorganisms like *Bacillus cereus*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and *Penicillium spp*. highlights their potential as natural preservatives in the food industry. This is particularly important given the increasing demand for natural and safe alternatives to synthetic preservatives, which are often associated with health risks.

The practical application of *Xylopia aethiopica* extracts in preserving fresh vegetables not only extends the shelf life of these products but also helps to reduce food waste and economic losses associated with spoilage. The sensory evaluation results indicate that these extracts do not adversely affect the taste, smell, or appearance of the vegetables, and in some cases, they may even enhance these qualities. This is a crucial consideration for consumer acceptance, as maintaining the sensory attributes of food products is essential for marketability. Thhe significant reduction in spoilage microorganisms achieved through the use of *Xylopia aethiopica* extracts has important public health implications. By effectively controlling pathogens like *Bacillus cereus* and *Escherichia coli*, these extracts help to mitigate the risk of foodborne illnesses, which are a major concern associated with the consumption of contaminated fresh produce . Additionally, the inhibition of mycotoxin-producing fungi like *Aspergillus niger* reduces the potential for mycotoxin exposure, thereby enhancing food safety (Karioti et al., 2004).

This study underscores the potential of *Xylopia aethiopica* extracts as a viable natural alternative for preserving fresh vegetables. The extracts not only inhibit microbial growth but also preserve or enhance the sensory qualities of the vegetables, making them suitable for practical application in food preservation. Given the growing consumer preference for natural preservatives, the use of *Xylopia aethiopica* extracts could play a significant role in meeting this demand while also contributing to food safety and sustainability (Ogbonna et al., 2013). Future research should focus on optimizing the extraction and application methods to maximize the efficacy of these extracts, as well as exploring their potential use in preserving other food products.

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