

ANTIMICROBIAL SCREENING OF ADIAN FRUIT (*TETRAPLEURA TETRAPTERA*) AND ATAIKO (*XYLOPIA AETHIOPICA*) AGAINST SELECTED ENTERIC BACTERIA

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ABSTRACT

Antimicrobial resistance is currently a world health challenge that have contributed to increased morbidity and mortality especially in low-income countries. Therefore, the aim of this research is to explore the antimicrobial potential of adian fruit [*tetrapleura tetraptera*) and ataike (*Xylopi aethiopia*) against selected microbes from clinical specimens (*Escherichia coli*, *Klebsiella sp.* and *Salmonella sp.*) commonly associated with increased antimicrobial resistance. The selected microbes were screened using cultural and biological methods. The results obtained showed that Ataike and Adian fruit had the highest zone of inhibition (mm) and mm against *Escherichia coli* and *Klebsiella sp.*, respectively. Findings from this study suggest that ataike and adian fruits possess notable antimicrobial properties against *Salmonella spp.*, *Klebsiella spp.* and *Escherichia coli*. Therefore, bioactive compounds contained in both fruits are potential sources of natural antimicrobial agents.

INTRODUCTION

In past few decades, the emergence of antimicrobial resistance has posed a significant global health challenge, necessitating the exploration of alternative sources of antimicrobial agents (Anesini and Perez, 2003). Natural products, particularly those derived from plants, have gained considerable attention for their potential to provide novel therapeutic options against infectious diseases (Cottiglia *et al.*, 2005; Adetumbi, Javor and Lau, 2004; Artizzu *et al.*, 2004; Alzoreky and Nakahara, 2003; Aktug and Karapinar, 2003). Adian fruit (*Tetrapleura tetraptera*) and ataike (*Xylopi aethiopia*), two traditionally used plants in various cultures, have been noted for their medicinal

properties, raising interest in investigating their potential antimicrobial activities. Aidan fruit and Ataike have a long history of traditional use in different parts of the world, particularly in Africa, for their therapeutic effects. These plants have been employed to treat a variety of ailments, including infections, digestive disorders, and pain (Akintola, Bodede and Ogunbamjo, 2015). The recognition of their medicinal properties in traditional medicine systems has prompted scientific inquiry into their bioactive constituents and potential health benefits. Both adian fruit and ataike are rich sources of phytochemicals, including alkaloids, flavonoids, terpenoids, and phenolic compounds. These bioactive constituents have

been associated with various biological activities, including antimicrobial properties (Akintola, Bodede and Ogunbamjo, 2015). While the exact mechanisms of action are often complex and multifaceted, these compounds are believed to disrupt microbial cell membranes, inhibit enzymatic processes, and interfere with microbial growth and proliferation (Brindha and Arthi, 2010; Arras and Grella, 2009; Igbinosa, Igbinosa, and Aiyegoro, 2009; Gonçalves *et al.*, 2008; Akgul and Kivanc, 2008; Artizzu *et al.*, 2004; Alzoreky, and Nakahara, 2003; Aktug and Karapinar, 2003). The overuse and misuse of conventional antimicrobial drugs have accelerated the development of antimicrobial resistance, rendering many existing treatments less effective (Bhatia and Bajaj, 1975). As a result, there is an urgent need to discover and develop novel antimicrobial agents that can combat infections caused by drug-resistant microorganisms (Akinpelu and Kolawole,

2004). Natural products, like those found in adian fruit and ataiko, offer a promising result for the development of alternative treatments treatment of infections caused by antibiotic resistant bacteria.

MATERIALS AND METHODS

Study area

The study was carried out in Yenagoa, Bayelsa State, Nigeria. Yenagoa boasts of lush mangroves, dense rainforests, and winding rivers. Yenagoa being a state capital, is a vibrant cultural center, predominantly inhabited by the Ijaw people. The city is a major player in Nigeria's oil and gas industry, contributing significantly to the nation's economy. Yenagoa's proximity to the Atlantic Ocean enriches its biodiversity, making it an ideal location for research on natural antimicrobial agents such as adian fruit and ataiko.



Figure 1: Adian fruit



Figure 2: Ataiko fruit

Bayelsa State is geographically situated at approximately 4.75°N to 5.45°N latitude and 5.25°E to 6.15°E longitude. This region offers a rich cultural and ecological context for academic and research pursuits.

Collection of samples

Adian fruit and ataiko samples were sourced from reputable and authentic sources known for providing high-quality, unadulterated natural products. Great care was taken to ensure the freshness and authenticity of the samples. The collection process involved working closely with local suppliers and expert botanists to ensure accurate identification and collection of the plant materials. Upon collection, the adian fruit and ataiko samples were carefully cleaned and prepared to eliminate any external contaminants. The plant parts used in the study were selected based on their traditional use and known bioactive properties. They were processed under controlled conditions to obtain the bioactive compounds for further analysis. Microorganisms used in the study were obtained from authenticated culture collections or clinical isolates. The selection of specific pathogens for testing was based on their relevance in public health, their association with infectious diseases, and their susceptibility profiles. Throughout the period of sample collection strict measures were followed to ensure aseptic techniques and prevent contamination of both plant and test organisms. Sterile tools and containers were used, and all procedures were carried out in a controlled laboratory environment to minimize the risk of external contamination.

Culture media

All bacteriological media used during the course of this work were prepared following the manufacturer's directions. The media used were MacConkey and nutrient agar.

Identification of isolates

Urine and stool samples collected from patients attending Toru-Orua Health Centre

were used for isolation of *Escherichia coli*, *Salmonella* spp. and *Klebsiella* spp.

Isolation of *Escherichia coli*

Isolating *Escherichia coli* from stool samples is a common practice in clinical and research settings, particularly when investigating gastrointestinal infections. The process involves several key steps to ensure accurate isolation and identification of the bacterium.

Firstly, sterile bottles were used for collection of stool samples, with aseptic conditions maintained in order to prevent contamination. Collected stool samples were transported to the microbiology laboratory, University of Africa, Toru-Orua, with the viability of the bacteria preserved during transit. 1 g of stool was diluted with 100 ml of water, mixed thoroughly and inoculated by streaking on MacConkey agar plates with the aid of a heat sterilized loop. The agar plates were incubated at 37°C, the optimal temperature for growth of *Escherichia coli*. The plates were left in the incubator for 24-48 hours, to allow the bacteria to grow. incubation, the agar plates were observed for bacterial growth. *E. coli* colonies on MacConkey agar are typically pink. Each of the pink colonies with distinct appearance were picked with a sterilized loop and sub-cultured on a fresh agar plate and incubated at 37°C for 24 hours. This process was repeated until pure colonies in a culture plate were obtained (Monica, 2009).

Isolation of *Salmonella* sp.

Isolation of *Salmonella* spp. from stool samples is a routine procedure in clinical microbiology, particularly when investigating gastrointestinal infections. Stool sample were collected in sterile bottles and transported to the Microbiology laboratory, University of Africa, Toru-Orua under aseptic conditions and preservation of bacterial viability. Collected samples underwent selective *Salmonella* enrichment by inoculation into Selenite Broth and by incubation at 37°C for 24 hours (Monica, 2009).

After enrichment, a portion of the broth was streaked onto Xylose Lysine Desoxycholate (XLD) agar and the plates were incubated at 37°C for 24-48 hours. *Salmonella* colonies on XLD agar typically appear red with a black center (Monica, 2009).

Isolation of *Klebsiella* sp

Isolating *Klebsiella* from urine samples is a routine procedure in clinical microbiology, particularly when investigating urinary tract infections (UTIs). Sterile universal plastic bottles were used to collect urine samples from patients. A urine sample was collected and transported immediately to the laboratory. The urine sample was inoculated onto the surfaces of MacConkey agar plates via streaking with a sterilized inoculating loop. The plates were incubated at 37°C for 24-48 hours to promote the growth of *Klebsiella* colonies (Monica, 2009).

After the incubation period, the agar plates were observed for bacterial growth. *Klebsiella* colonies on MacConkey agar may exhibit a pink colour (Monica, 2009).

Method of plant extraction

Selection and Preparation of Plant Material

Aidan fruit and ataiko seeds samples were carefully selected based on their quality, maturity, and authenticity. The plant parts used were inspected to ensure they were free from damage and contaminants. Upon collection, the plant materials were thoroughly cleaned to remove any extraneous matter. They were then air dried under controlled conditions for a period of 48 hours in order to preserve their bioactive components. Dried plant parts were ground or milled into a fine powder to increase the surface area, facilitating the extraction of bioactive compounds. Consistency in particle size was maintained to ensure uniform extraction. Extraction of bioactive compounds were carried out by soaking the plant materials in ethanol-water mixture in an air tight container for 24 hours. After filtration of solid residue, the bioactive components were

separated from the solvent using the rotary evaporator (Chibuye *et al.*, 2023). The resulting concentrated extracts were stored in airtight amber-colored vials from whence they were evaluated for their antimicrobial properties

Disc diffusion Method

Media preparation

Nutrient agar was prepared following manufacturer's instruction. Once prepared, the nutrient agar was poured into Petri dishes. The molten agar plates were allowed to solidify at room temperature.

Disc preparation

Filter paper was sterilized by autoclaving at 121 °C for 15 minutes at 15 psi, and thereafter impregnated with the plant extract. Subsequent handling of the filter paper was done using sterilized forceps (Monica, 2009; Bauer *et al.*, 2004).

Preparation of inoculum

A 24 h culture of *Salmonella* sp., *Klebsiella* sp. and *Escherichia coli* were suspended in 5 ml of sterile normal saline and turbidity adjusted to 0.5 MacFarland's standard which is an equivalent of 1×10^5 CFU/ml (Haque *et al.*, 2022; Maji, *et al.*, 2010; Monica, 2009; Bauer *et al.*, 2004; Espinel – Ingroff, *et al.*, 2002).

Preliminary antimicrobial screening of plant extracts

Exactly 0.5 ml of each of the inoculum was inoculated onto freshly prepared MacConkey agar plates. Each of the disc impregnated with plant extract was then transferred, using sterile forceps to the surface of the agar plate. The medium was incubated for 24 – 72 h at 37 °C (Haque *et al.*, 2022; Monica, 2009; Bauer *et al.*, 2004; Espinel – Ingroff, *et al.*, 2002).

RESULTS AND DISCUSSION

The zone of inhibition of ataiko, adian fruit and the control, against the test isolates are depicted in Table 1, 2 and 3, respectively.

Table 1: Zone of inhibition of ataiko against *Escherichia coli*, *Salmonella* and *Klebsiella* spp

Organism	Concentration of plant extract	Zone of inhibition			Mean (cm)
		24hrs	48hrs	72hrs	
<i>Escherichia coli</i>	100%	0.4cm	0.9cm	0.9cm	22/3= 0.73cm
<i>Klebsiella</i> spp.	100%	No clearance	No clearance	No clearance	0/3= 0cm
<i>Salmonella</i> spp.	100%	No clearance	No clearance	No clearance	0/3=0cm

The findings, as is shown in Table 1, underscore the promising antimicrobial properties of ataiko against *E. coli* and Aidan Fruit against *Klebsiella* spp. that justifies its use for the development of natural antimicrobial agents. Future studies should delve into the identification and characterization of specific bioactive

compounds present in the extracts responsible for antimicrobial properties (Daillo *et al.*, 2011). This research has contributed to the growing body of knowledge on the potential applications of plant extracts as alternatives to synthetic substances to control the growth of pathogenic organisms responsible for infections in humans.

Table 2: Zone of inhibition of adian fruit against *Escherichia coli*, *Salmonella* and *Klebsiella* spp.

Organism	Concentration of plant extract	Zone of Inhibition			Mean (cm)
		24hrs	48hrs	78hrs	
<i>Escherichia coli</i>	100%	No clearance	No clearance	No clearance	0/3 = 0
<i>Klebsiella</i> spp.	100%	0.5cm	0.5cm	0.5cm	1.5/3 = 0.5
<i>Salmonella</i> spp.	100%	No clearance	No clearance	No clearance	0/3 = 0

The result presented in Table 2 shows that adian fruit extract has no inhibitory effect against *Escherichia coli*. Nonetheless, a consistent zone of inhibition of 0.5cm against *Klebsiella* spp. was noted over 24hrs, 48hrs, and 78hrs, indicating a potential antimicrobial activity of adian fruit extract against the test isolates. Aidan fruit extract did not show observable antimicrobial activity against

Klebsiella spp. This is similar with the result that involved the use of ataiko extract. Aidan fruit showed no observable inhibition against *Salmonella*. The mean zone of inhibition of the plant extracts against the test isolates have provided valuable information on the antimicrobial potential of the plant extracts (Patel, Venugopalan and Pradeep, 2007).

Table 3: Zone of inhibition of ampiclox (control) against *Escherichia coli*, *Salmonella* and *Klebsiella* spp.

Organism	100 mg/ml ampiclox	Zone of Inhibition			Mean (cm)
		24hrs	48hrs	72hrs	
<i>Escherichia coli</i>	100%	1.1cm	1.1cm	1.1cm	3.3/3=1.1cm
<i>Klebsiella</i> spp	100%	1cm	1.1cm	1.1cm	3.2/3 = 1.1cm
<i>Salmonella</i> spp.	100%	1.2cm	1.2cm	1.3cm	3.7/3=1.23cm

The result presented in Table 3 shows the zone of inhibition of ampiclox against *Escherichia coli*, *Salmonella* and *Klebsiella* spp. The result shows that ampiclox had a uniform zone of inhibition against *Escherichia coli* at the time intervals of exposure to the bacterium. With regards to antimicrobial activity of ampiclox against *Klebsiella* sp. and *Salmonella* spp. the antibiotic showed increasing zone of inhibition at the various time intervals of exposure to the test organisms.

DISCUSSION

The results obtained from this study has shown that adian fruit (*Tetrapleura tetraptera*) and ataiko (*Xylopia aethiopica*) extract demonstrated antimicrobial activity against *Klebsiella* spp. and *Escherichia coli*, respectively. Interestingly, the result showed that ampiclox possess antimicrobial properties against *Escherichia coli*, *Salmonella* spp and *Klebsiella* spp. which is in agreement with the report of Anesimi and Perez (2003) and Mahesh and Satish (2008) (Anesini and Perez, 2003; Mahesh., and Satish, 2008). The mean zone of inhibition of 0.73cm further supports antimicrobial activity of *Xylopia aethiopica* against *E. coli* which is in line with the reports of Yiadom, and his Co-workers (Yiadom, Fiagbe and Ayim, 2012). However, there was no observable zone of inhibition for the plant extract against *Klebsiella* spp. and *Salmonella* spp. resulting in a mean zone of inhibition of 0 cm.

In the case of *Tetrapleura tetraptera* extract, it demonstrated no observable inhibitory effect against *E. coli*. Nonetheless, a zone of inhibition of 0.5cm against *Klebsiella* spp. was noted at 24, 48 and 72 h, indicating potential antimicrobial activity. Both adian fruit and ataiko extract showed no observable inhibition against *Salmonella* spp. The mean zone of inhibition of adian fruit and ataiko extracts against *Klebsiella* spp. and *Escherichia coli*, respectively have provided useful information on the antimicrobial potential of the plants. Therefore, both plants extracts could be used in the development of natural antimicrobial

agents (Patel, Venugopalan and Pradeep, 2007).

Further studies to identify and characterize specific bioactive compounds in ataiko and adian fruits is strongly advocated.

CONCLUSION

The investigation into the antimicrobial properties of adian fruit and ataiko against *Escherichia coli* *Klebsiella* spp. and *Salmonella* spp. yielded promising results, demonstrating significant inhibitory effects on bacterial growth. The positive outcomes from in vitro assays has highlighted the potential importance of these natural extracts in antimicrobial research. To leverage these findings, future efforts should focus on formulating topical applications, conduct in vivo studies for therapeutic validation, and exploring synergies with established antibiotics. These strategic recommendations aim to bridge the gap between laboratory findings and practical medical applications, offers a promising pathway for further research and development. The study has contributed valuable insights to the field of natural antimicrobial agents, suggesting the potential of adian fruit and ataiko in innovative approaches for combating bacterial infections.

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