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Assessment of the Antimicrobial Potency of Unripe Plantain Fruits and Peelings Extracts against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*

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ABSTRACT: The objective of this paper was to assess the antimicrobial potency of unripe plantain fruits and peelings extracts against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* using disc diffusion technique, with ciprofloxacin as control. The Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) were determined using 100 mg/ml, 75 mg/ml, 50 mg/ml and 25 mg/ml concentrations of each extract. Phytochemicals detected in the extracts were flavonoids, glycosides, tannins, alkaloids and phenol. All isolates were susceptible to the extract at the highest concentration of 100 mg/ml. At the lowest concentration of 25 mg/ml, *E. coli* showed resistance, while *Salmonella typhi* and *Staphylococcus aureus* showed intermediate resistance to the extract. The MIC and MBC of the extract on the test organisms were 14 and 20 mg/ml for *E. coli*; 25 and 16 mg/ml for *Salmonella typhi* whereas it was 100 mg/ml and 15 mg/ml for *Staphylococcus aureus*. *E. coli* was more susceptible to the extract and *Staphylococcus aureus* was the least. The results revealed the presence of phytochemicals with antimicrobial properties against *E. coli*, *Staphylococcus* sp. and *Salmonella* sp. Thus, unripe plantain fruits and peelings could be harnessed for the control of *E. coli*, *Staphylococcus aureus* and *Salmonella* sp., as natural antibiotics.

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Medicinal plants are commonly used as remedies for many diseases, as they are considered alternative therapeutic tools in healthcare (Alese *et al.*, 2017). Medicinal plants contain bioactive compounds, with a broad range of functions including killing of microorganisms and inhibition of microbial activities (Albertyn *et al.*, 2015; Hosseinzadeh *et al.*, 2015; Jaiswal *et al.*, 2016). The utilization of medicinal plants is a typical practice in many societies all over world, although more popular in African and Asian societies. The modern crave for organic products has aided the widespread acceptance in the developed nations, even as research are beginning to point to the efficacy of medicinal plants (Ajiboye *et al.*, 2018). Medicinal plant with antimicrobial properties are gaining considerable interest as a result of their effectiveness, low cost and the availability, high cost of orthodox medicines, inadequacy of modern health care and above all, as a result of the safety of synthetic drugs and resistance to antimicrobial agents (Verpoorte, 2017; Okaba *et al.*, 2022).

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Antibiotics revolutionized modern health and they are regarded as greatest achievements in medicine in modern time. Nevertheless, as microorganisms began to develop resistance to antibiotics, their suitability for sustainable management of infectious disease is now being questioned. With the advent of antibiotics resistance by disease-causing microorganisms, attention is shifting to alternative medicine. Thus, traditional medicine, once relegated as folk medicine, is now making a resurgence (Verpoorte, 2017; Amaeze *et al.*, 2018). Indeed, antibiotic resistance and perceived safety of synthetic drugs are key reasons for their acceptance in the developed world (Zhang *et al.*, 2015), although acceptance in the developing nations is mainly due to cost, limited and restricted access to orthodox medicine (WHO, 2013). Acceptance of the use of herbal medicine worldwide is chiefly because of its natural origin (Zhang *et al.*, 2015; Chatfield *et al.*, 2018).

Herbal medicine or phytomedicine exploits the medicinal potency of roots, stems, herbs, nectar, rhizome, barks and any plant part that can be applied for therapeutic purposes (Bruno *et al.*, 2018; Chatfield *et al.*, 2018; Shakib *et al.*, 2019). The use of plants in folk medicine is not new and their perceived health benefit have been well documented (Rojas *et al.*, 2006; Shodehinde *et al.*, 2015; Amutha and Selvakumari, 2016; Asuquo and Udobi 2016).

Plantain (*Musa paradisiaca*), a major food crops in some parts of Africa, is used in its unripe form in Nigeria mainly for the management of diabetes mellitus (Agarwal *et al.*, 2009; Eleazu *et al.*, 2013; Famakin *et al.*, 2016; Jaiswal *et al.*, 2016). Because plantain serves dietary and nutritional needs, its curative function has not been fully explored.

There are however, few reports on the antimicrobial activities of plantain against several microorganisms including bacteria, fungi and viruses. Plantain has been reported as efficacious for wound healing (Shodehinde *et al.*, 2015) and bactericidal against species of Escherichia and Staphylococcus (Asoso *et al.*, 2016).

The therapeutic efficacy of plantain has been reported to be on account of its phytochemicals including flavonoid, glycosides and phenolic compounds (Agarwal *et al.*, 2009; Eleazu *et al.*, 2013; Famakin *et al.*, 2016; Amutha and Selvakumari, 2016).

Hence, the objective of this paper was to assess the antimicrobial potency of unripe plantain fruits and peelings extracts against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* using disc diffusion technique, with ciprofloxacin as control.

MATERIALS AND METHODS

Collection of plantain samples: The unripe peels and fruits were obtained from fresh unripe plantains purchased from Market Square, Ekpoma, Edo State, Nigeria. The peels and fruits were dried and ground to powder using a milling machine (Silver Crest D-P2030D).

Collection of test bacteria: The tests isolates viz. *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* were obtained from Irrua Specialist Teaching Hospital, Irrua, Edo State. The organisms were collected on sterile agar slants and incubated at 37℃ for 48hours in the laboratory, and preserved in the refrigerator at 4ºC. The organisms were identified by their colony morphology using Nutrient Agar for *Staphylococcus aureus*, Eosin Methylene Blue for *Escherichia coli*, and Blood Agar for *Salmonella typhi* and by their biochemical profile upon Gram staining, catalase, oxidase, Voges-Proskeuer, methyl-red, citrate, sugar fermentation and motility tests.

Extraction of plantain peel and fruit: The extraction was carried out by steeping in ethanol, as depicted by Doherty *et al.* (2010) with slight changes. Forty grams (40g) of the ground sample was soaked in 100ml of ethanol for 3 days, and thereafter filtered with muslin cloth and further filtered using Whatman filter paper. The filtrate was concentrated in a rotary evaporator. A stock concentration of the extract was prepared by dissolving 4g of extract in 40ml of Dimethyl sulfoxide (DMSO), from which the different concentrations used were calculated.

Antibiotic Susceptibility Test: Different concentrations (25, 50, 75 and 100mg/ml) of the ethanol extracts were prepared with sterile distilled water in sterile test tubes. Standardized concentrations (McFarland 0.5) of overnight cultures of test isolates were aseptically swabbed on agar plates. Sterile discs impregnated with the 25, 50, 75 and 100mg/ml of the extracts and were gently placed on the appropriately labeled plates. Standard antibiotic discs containing Ciproflox (10µg) served as control. Plates were incubated at 37°C 24hours. Clear zones around the discs were measured to ascertain the degree of susceptibility of the test bacteria.

Determination of Minimum inhibitory Concentration and Minimum Bactericidal Concentration: The MIC of the extracts was determined as per the method of Adebayo *et al.* (1989). Double-folds serial dilutions were prepared with the extract in nutrient broth and inoculated with test isolates. The culture was incubated at 37℃ for 48hours. The smallest concentration at which growth was inhibited was taken as the MIC. The MBC was determined as the lowest concentration of the extract that killed the test organisms.

Phytochemical Screening of Extracts: Qualitative phytochemical screening of the extracts for the presence of tannins, flavonoids, alkaloid, glycosides and phenol, followed the procedures described by Shah *et al.* (2014).

RESULTS AND DISCUSSION

The results of this study showed that all the phytochemicals (tannins, flavonoids, alkaloid, glycosides and phenol) tested were all detected in the extract. Table 1 shows the morphology, gram reaction and biochemical characteristics of test isolate. The results confirmed the test isolates as *E. coli*, *Salmonella typhi* and *Staphylococcus aureus.* The antibacterial activity of the plant extracts is presented on Tables 2. All isolates were susceptible to the extract at the highest concentration of 100 mg/ml. At the lowest concentration of 25 mg/ml, *E. coli* showed resistance, while *Salmonella typhi* and *Staphylococcus aureus* showed intermediate resistance.

Table 3 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the unripe plantain fruits and peelings extracts on the test organisms. The MIC and MBC were 14 and 20 mg/ml for *E. coli*; 25 and 16 mg/ml for *Salmonella typhi* whereas it was 100 mg/ml and 15 mg/ml for *Staphylococcus aureus*. *E. coli* was more susceptible to the extract and *Staphylococcus aureus* was the least.

Table 1: Characteristics of test bacteria

Cultural characteristics	Gram reaction/ Morphology	MOT	OXI	IND	CIT	CAT	URE	COA	GLU	MAL	SUC	LAC	
Round pinkish colonies	Gram -ve rod	+	-	+	-	-	-	-	A/G	A/G	A	A/G	*E. coli*
Colorless with black center	Gram -ve rod	+	-	-	-	-	-	-	+	+	-	-	*Salmonella typhi.*
Smooth, round golden yellow cluster	Gram +ve non Spore forming rod	-	-	-	+	+	-		A	A	A	A	*Staphylococcus aureus*

Key: Mot: Motility; Oxi: Oxidase; Ind: Indole; Cit: Citrate; Cat: Catalase; Ure: Urease; Coa: Coagulase; Glu: Glucose, Mal: Maltose, Suc: Sucrose; Lac: Lactose;+: Positive; -: Negative ; A/G: Acid and Gas Production; A: Acid production

Table 2: Antibiotic susceptibility test of ethanol extract of unripe plantain fruits and peelings against test bacteria

Test isolate	**Extract Concentration (mg/ml)**			
	100	75	50	25
Escherichia coli	27mm	25mm	22mm	3mm
Salmonella typhi	26mm	26mm	19mm	14mm
Staphylococcus aureus	26mm	18mm	18mm	15mm

Range: Resistance<13; Intermediate-14 to 17; Sensitive-17 and above

Table 3: Minimum inhibitory and minimum bactericidal concentrations of ethanol extract of unripe plantain against test bacteria

Test isolate	**Extract Concentration (mg/ml)**	
	MIC	MBC
Escherichia coli	14	20
Salmonella typhi	25	16
Staphylococcus aureus	100	15

In this study the antimicrobial effect of the ethanol extract of unripe plantain fruits and peelings was determined. The antimicrobial properties of plant extracts have been attributed to the presence of secondary metabolites (Ugboko *et al.*, 2020). The results of phytochemicals analysis of unripe plantain fruits and peelings extract showed that all the phytochemicals (tannins, flavonoids, alkaloid, glycosides and phenol) screened were present. These phytochemicals are commonly reported phytochemicals in plantain. Abdullah *et al.* (2024) characteried the phytochemicals in plantain to include tannins, flavonoids, alkaloid, glycosides, phenol, as well as steroids and saponins. Shodehinde and Oboh (2013) reported phenol and flavonoid as the main aqueous extracts of unripe plantain responsible for antioxidant activity monitored in rat. All test isolates (*E. coli*, *Salmonella typhi* and *Staphylococcus aureus*) were found to be susceptible to the extract at concentrations around 50-100 mg/ml. The extract showed more activity against the Gram negative bacteria (*E. coli* and *Salmonella typhi*) at concentrations of 50 and 75 mg/ml. However, at the lowest concentration of 25 mg/ml, *E. coli* was resistant to the extract, while *Salmonella typhi* and *Staphylococcus aureus* showed intermediate results. Our results are in contrast with findings of other researchers (Okaba *et al.*, 2022) that reported that most plant extracts have more inhibitory effect on Gram positive bacteria than Gram negative bacteria. Phytochemicals of unripe plantain fruits and peelings also showed activities against pathogenic bacterial strains in other studies including Gram positive and Gram negative bacteria (Abdullah *et al.*, 2024). The inhibitive effect of plant extracts are dependent on active phytochemical compounds present in them (Immanuel *et al.*, 2013; Okaba *et al.*, 2022). In the present study tannins, flavonoids, alkaloid, glycosides and phenolic were detected in the extract, which could explain the activity of the extract on the test organism.

The findings of this study is in agreement with the report by Asoso *et al.* (2016) with sensitivity of 0, 10 and 9 mm (plantain fruit ethanol extract) and 27, 17 and 17 mm (plantain peel ethanol extract) for *E. coli*, *Salmonella typhi* and *Staphylococcus aureus* respectively, which confirms that unripe plantain fruits and peels contain antimicrobial compounds. The sensitivity of isolates in the present study was higher than reported by Asoso *et al.* (2016), perhaps because the present student used a mixture of both the fruit and the peel of the plant. Again, plants could differ in their phytochemical composition both in types and concentration, owing to the growing condition and the extraction method, which could explain the high susceptibility of both Gram positive and Gram negative bacteria in this study compared to other studies.

The MIC and MBC of the ethanol extract of unripe plantain peels and fruits were 14 and 20 mg/ml for *E. coli*; 25 and 16 mg/ml for *Salmonella typhi* whereas it was 100 mg/ml and 15 mg/ml for *Staphylococcus aureus*. *E. coli* and *Salmonella typhi* were more susceptible to the extract at lower concentration and *Staphylococcus aureus* was susceptible at higher concentration. This suggest that lower concentrations of the extract would be needed for the control of *E. coli* and *Salmonella typhi*, which is very good, given that Gram negative bacteria have been reported to be less susceptible to plant extract. However, the MIC and MBC reported in this study are lower than values reported Asoso *et al.* (2016) for all isolates. In general, plant materials with antimicrobial properties can cause granular cytoplasm, cytoplasmic membrane rupture, and inactivation or inhibition of enzymes activity at sufficiently high concentrations relative to synthetic antibiotics (Iwu *et al.*, 1999; Ugboko *et al.*, 2016). The advantage in the use of natural antibiotics from plant materials is in their safety and low resistance (Okaba *et al.*, 2022).

Conclusion: This study has demonstrated that the ethanol extract of unripe plantain contains phytochemical with bioactive function as clinical isolates (*E. coli, Salmonella typhi* and *Staphylococcus aereus*). The specific phytochemical compounds exhibiting the antimicrobial effects on the test isolates need to be identified and utilized in drug formulation against pathogenic microorganisms.

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