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Antibacterial Activities of Plantain (*Musa paradisiaca*) Peel and Fruit

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ABSTRACT

Plantain (*Musa paradisiaca*) is used as a medicinal plant employed in traditional system of healing diverse diseases such as hepatitis, skin infections, problems concerning the digestive organs, respiratory organs, reproduction, the circulation, anti-inflammatory, antiviral, analgesic, antioxidant, anti-carcinogenic, antitumor, anti-nociceptive (reducing the sensitivity to painful stimuli), weakly antibiotic, immune modulation, anti-ulcerogenic, anti-leukemic and antihypertensive effects, and for reducing fever. The antimicrobial activities of methanol, ethanol and acetone extracts of *M. paradisiaca* peel and fruit were tested in-vitro against seven typed Gram negative and positive pathogenic bacteria (*Salmonella typhi* 22648 ATCC, *Salmonella typhi* 23456 ATCC, *Escherichia coli* 35218 ATCC, *Shigella dysenteriae* 24162 ATCC, *Klebsiella pneumonia* 34089 ATCC, *Staphylococcus aureus* 25923 ATCC and *Bacillus subtilis* 21332 ATCC). The clinical isolates are *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. The antibacterial activity was assessed by agar well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. *M. paradisiaca* peel methanol and ethanol extract showed a higher zone of inhibition of test organisms than *M. paradisiaca* fruit methanol and ethanol extract which could be due to phytochemical constituents. Phytochemical analysis of the peel and fruit indicated the presence of alkaloid, flavonoids, saponins, tannins, phlobatannins, glycoside, and terpenoids. *M. paradisiaca* acetone extract from both fruit and peel showed no antibacterial activities towards the organisms used.

Keywords: Antibacterial, clinical bacteria, *Musa paradisiaca*, inhibition

INTRODUCTION

Medicinal plants, as source of remedies, are widely used as alternative therapeutic tools for the prevention or treatment of many diseases [1]. Plants are a viable, unlimited source of bioactive molecules, including antimicrobial agents which protect them from microorganism, insects, and predators [2, 3, 4]. The use of medicinal herbs in traditional system of medicine is a common practice in many cultures around the world especially in African societies. This practice has gained widespread acceptance in developing as well as in developed nations. Researchers are also beginning to appreciate the role of medicinal plants in health care delivery [5]. In recent time, interest with herbal medicine for antimicrobial activities has been increased significantly. This is as a result of the effectiveness, low cost and the availability of these herbal medicines, the economic crisis, high cost of industrialized medicines, inefficient public access to medical and pharmaceutical care, in addition to the side effects caused by synthetic drugs are some of the factors contributing to the central role of medicinal plants in health care [6, 7].

Plantain (*Musa paradisiaca*) is a major food crops in the humid and sub-humid parts of Africa where its starchy fruits are generally cooked or fried before consumption and serves as major sources of energy for millions of people

in these regions [8]. It belongs to the natural order, plantaginaceae which contains more than 200 species, twenty-five or thirty of which have been reported. The common plantain has broad, irregular oval leaves, abruptly contracted at the base into a long broad, channelled foot stalk. The fully grown blade is 1.3–2.4 meters long and about two- third as broad, usually smooth, with several parallel veins. Plantain grows more than any other plant in compacted soils, is abundant beside paths, roadside and other areas with frequent soil compaction. It is also common in grassland and as a weed among crops.

Since unripe plantain flour is used by the traditional medical practitioners in Nigeria for dietary management of diabetes mellitus and other disease conditions, this study is therefore aimed at investigate the antibacterial actives of unripe plantain flour derived from fruits and peels.

MATERIALS AND METHODS

Collection of plantain samples

Fresh unripe plantains were obtained from Garki market, Abuja, Nigeria. The peel and fruit (unripe) were removed by hand and cut into smaller pieces for easy drying. The dried peel and fruit were ground using a milling machine. The powdery samples were packed into screwed bottles and labelled appropriately.

Collection of test organisms (bacterial strains)

Test bacterial strains used in the study are clinical and typed isolates were kindly provided by the microbiology department in National Institute of Pharmaceutical Research and Development (NIPRID) Abuja, Nigeria. The test isolates are *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* (25923), *Bacillus subtilis* (21332), *Escherichia coli* (35218), *Samonella typhi* (22648), *Shigella dysentrariae* (24162), *Salmonella typhi* (23456), and *Klebsiella pneumonia* (34089).

Extraction of plantain peel and fruit

One hundred and twenty grammes of each powder were extracted with methanol, ethanol and acetone for 72 hours with intermittent shaken in a shaking water bath. It was filtered through Whatmann No filter paper. The extracts were evaporated under reduced pressure at 45 °C using a rotary evaporator.

Antibacterial Activities of extracts

One millilitre of each test isolate prepared to McFarland standard was aseptically pour plated with freshly prepared Mueller-Hinton agar. The seeded plates were stand for 2 hours before wells bored into the agar using a sterile cork borer. The residual extracts were dissolved in 5% Dimethyl Sulphur Oxide (DMSO⁴). Thus, 100µl of the different extract were placed into the wells and the plates were incubated at 37 °C for 24 – 48 hours. Antibacterial activity of extracts was evaluated by measuring the diameter of circular inhibition zones around the well. Tests were performed in triplicate.

Antibiotic Susceptibility Test

The antibiotic susceptibility test was done using the agar-disc diffusion method. Molten Mueller Hinton agar cooled to about 45 °C was poured into sterile petri dishes. And 1 ml of cell suspensions prepared in comparison to 0.5 McFarland standard was added and spread evenly onto the surface of the agar plates using sterile swap sticks. The antibiotic disc was placed aseptically on the agar surface (the positive disc for the gram positive and the negative disc for the gram negative) and plates were incubated at 37 °C for 24 hours. The zone of inhibition was then measured and recorded. The tests were done in duplicate to ensure reliability [9].

Minimum Inhibitory Concentration (MIC) and minimum bactericidal Concentration (MBC)

Minimum inhibitory concentration (MIC) was determined for each extract showing antimicrobial activity against the test isolates using broth micro dilution method. The MIC values were taken as the lowest concentration of the extracts in the well of the test tube that showed no turbidity after incubation. Turbidity of the wells was interpreted as visible growth of organisms. The minimum bactericidal concentration (MBC) was determined by sub culturing from each well showing no growth. Least concentration of the extract showing no visible growth on sub culturing was taken as MBC [10].

RESULTS

All the test organisms were susceptible to the solvent extracts of *M. paradisiacal* peel and fruit. (Tables 1a and 1b). Among the solvent extraction, methanol extract was the most potent on the test organisms followed by the ethanol and acetone extracts. However, the most inhibited test isolates include *Salmonella typhi*, *Bacillus subtilis*, *Escherichia coli*, and *Shigella dysenteriae*, which were inhibited with 35, 37, 40, and 30 mm by the methanol extract of plantain peel and 27 and 22 mm by ethanol extract of peel while the acetone extract had little or no effect on the organisms. These organisms were also inhibited by the fruit extracts of methanol with 27, 27, 20 and 27mm while the ethanol and acetone extracts had little or no effect on test organisms respectively. The inhibition on the test organisms by the solvent extracts of plantain peel and fruit are comparable to the inhibition by both positive and negative control antibiotic (Figures 1 and 2). Figure 1 and 2 expresses susceptibility of test organisms to commercial antibiotic (positive and negative control) among the commercial antibiotics inhibited. It was observed that all the Gram positive organisms were resistant to ceftazidime, augmentin, cloxacillin, and ofloxacin while *Staphylococcus aureus* (clinical isolate) was resistant to erythromycin and *Bacillus subtilis* (ATCC 21332) was resistant to cefuroxime. All Gram negative organisms were resistant to cefuroxime, and augmentin, *Escherichia coli* (ATCC 35218) was resistant to ceftazidime, cefuroxime, gentamicin, cefixime, ofloxacin, augmentin, nitrofurantoin, and ciprofloxacin. *Shigella dysenteriae* (ATCC 24162) was resistant to ceftazidime, cefuroxime, and augmentin. In comparison of the plant extract with commercial antibiotics, the test isolates in most cases were highly susceptible to the plant extract than the commercial antibiotics.

Table 1a: Antibacterial activities of the peel of *Musa paradisiacal* Zone of inhibition (mm)

Organisms	Methanol	Ethanol	Acetone
<i>Escherichia coli</i>	40 ± 0.0	27 ± 0.0	12 ± 0.0
<i>Escherichia coli</i> ATCC 35218	22 ± 0.0	17 ± 0.0	-
<i>Staphylococcus aureus</i>	22 ± 0.0	17 ± 0.0	12 ± 0.0
<i>Staphylococcus aureus</i> ATCC 25923	22 ± 0.0	17 ± 0.0	-
<i>Salmonella typhi</i>	35 ± 0.0	27 ± 0.0	-
<i>Salmonella typhi</i> ATCC 22648	27 ± 0.0	17 ± 0.0	-
<i>Salmonella typhi</i> ATCC 23456	17 ± 0.0	22 ± 0.0	-
<i>Shigella dysenteriae</i> ATCC 24162	30 ± 0.0	22 ± 0.0	12 ± 0.0
<i>Klebsiella pneumonia</i> ATCC 34089	27 ± 0.0	17 ± 0.0	7 ± 0.0
<i>Bacillus subtilis</i> ATCC 21332	37 ± 0.0	27 ± 0.0	10 ± 0.0

Table 1b: Antibacterial activities of the fruit of *Musa paradisiacal* Zone of inhibition (mm)

Organisms	Methanol	Ethanol	Acetone
<i>Escherichia coli</i>	20 ± 0.0	-	-
<i>Escherichia coli</i> ATCC 35218	17 ± 0.0	22 ± 0.0	-
<i>Staphylococcus aureus</i>	22 ± 0.0	-	-
<i>Staphylococcus aureus</i> ATCC 25923	22 ± 0.0	9 ± 0.0	-
<i>Salmonella typhi</i>	27 ± 0.0	10 ± 0.0	-
<i>Salmonella typhi</i> ATCC 22648	27 ± 0.0	17 ± 0.0	-
<i>Salmonella typhi</i> ATCC 23456	22 ± 0.0	17 ± 0.0	-
<i>Shigella dysenteriae</i> ATCC 24162	27 ± 0.0	-	-
<i>Klebsiella pneumonia</i> ATCC 34089	7 ± 0.0	-	-
<i>Bacillus subtilis</i> ATCC 21332	27 ± 0.0	17 ± 0.0	7 ± 0.0

Table 2a: Determination of minimum inhibition concentration (MIC) of *Musa paradisiacal* peel extract

Organisms	Methanol	Ethanol	Acetone
<i>Escherichia coli</i>	150	200	-
<i>Escherichia coli</i> 35218 ATCC	150	200	-
<i>Salmonella typhi</i>	150	250	-
<i>Salmonella typhi</i> 22648 ATCC	150	250	-
<i>Salmonella typhi</i> 23456 ATCC	150	200	-
<i>Staphylococcus aureus</i>	200	200	-
<i>Staphylococcus aureus</i> 25923 ATCC	200	150	-
<i>Shigella dysenteriae</i> 24162 ATCC	150	200	-
<i>Klebsiella pneumonia</i> 34089 ATCC	200	250	-
<i>Bacillus subtilis</i> 21332 ATCC	100	200	-

Table 2b: Determination of minimum inhibition concentration (MIC) of *Musa paradisiaca* Fruit extracts (mg/ml)

Organisms	Methanol	Ethanol	Acetone
<i>Escherichia coli</i>	200	250	–
<i>Escherichia coli</i> 35218 ATCC	200	250	–
<i>Salmonella typhi</i>	150	300	–
<i>Salmonella typhi</i> 22648 ATCC	200	300	–
<i>Salmonella typhi</i> 23456 ATCC	200	250	–
<i>Staphylococcus aureus</i>	250	250	–
<i>Staphylococcus aureus</i> 25923 ATCC	250	200	–
<i>Shigella dysenteriae</i> 24162 ATCC	200	250	–
<i>Klebsiella pneumonia</i> 34089 ATCC	250	200	–
<i>Bacillus subtilis</i> 21332 ATCC	150	200	250

Table 3a: Determination of Minimum Bactericidal Concentration (MBC) of peel extract (mg/ml)

Organisms	Methanol	Ethanol	Acetone
<i>Escherichia coli</i>	200	250	–
<i>Escherichia coli</i> 35218 ATCC	200	250	–
<i>Salmonella typhi</i>	250	300	–
<i>Salmonella typhi</i> 22648 ATCC	250	300	–
<i>Salmonella typhi</i> 23456 ATCC	200	250	–
<i>Staphylococcus aureus</i>	200	250	–
<i>Staphylococcus aureus</i> 25923 ATCC	200	250	–
<i>Shigella dysenteriae</i> 24162 ATCC	250	–	–
<i>Klebsiella pneumonia</i> 34089 ATCC	300	–	–
<i>Bacillus subtilis</i> 21332 ATCC	200	250	–

Table 3b: Determination of minimum bactericidal concentration (MIC) of fruit Extract(mg/ml)

Organisms	Methanol	Ethanol	Acetone
<i>Escherichia coli</i>	300	–	–
<i>Escherichia coli</i> 35218 ATCC	250	250	–
<i>Salmonella typhi</i>	200	250	–
<i>Salmonella typhi</i> 22648 ATCC	200	250	–
<i>Salmonella typhi</i> 23456 ATCC	200	250	–
<i>Staphylococcus aureus</i>	250	–	–
<i>Staphylococcus aureus</i> 25923 ATCC	250	300	–
<i>Shigella dysenteriae</i> 24162 ATCC	300	–	–
<i>Klebsiella pneumonia</i> 34089 ATCC	250	300	–
<i>Bacillus subtilis</i> 21332 ATCC	200	250	–

The highest sensitivity exhibited on the test organisms was 40 mm with methanol extract of the least inhibition was 7 mm with acetone extract. However, the highest inhibition exhibited by commercial antibiotic on test solates was 30 mm by gentamicin and ofloxacin and least inhibition of 7 mm by cefuroxime.

The minimum inhibitory concentration (MIC) of the extracts ranged from 50 – 300 mg/ml as shown in Table 2a and b. It was observed that at higher concentration there was a stronger activity against test organisms. There was no activity at lower concentration against the test isolates. The result obtained ascertained 100-300 mg/ml as MIC value for plantain peel and 200-300 mg/ml for plantain fruit extracts. The MIC value for plantain peel methanol extract on *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* (clinical and typed isolates) and *Bacillus subtilis* (ATCC 21332) were between 100 - 200 mg/ml while the ethanol extract was valued at 200 mg/ml on each of this isolates. The MIC value of fruit extract on *Escherichia coli* (clinical and typed isolates) and *Shigella dysenteriae* (ATCC 24162) was 200 and 250 mg/ml for methanol and ethanol extract and *Bacillus subtilis* (ATCC 21332) with 150 and 200 mg/ml for methanol and ethanol extract respectively.

The minimum bactericidal concentration (MBC) of the extract was evaluated between 50 – 300mg/ml as shown in table 3a and b. The MBC value for methanol and ethanol peel extract on *Escherichia coli* 35218 ATCC and clinical isolate, *Staphylococcus aureus* 25923 ATCC and *S. aureus*, *Salmonella typhi* 22648 ATCC and *S. typhi* and *Bacillus subtilis* 21332 ATCC were 200 and 250mg/ml respectively, MBC value for methanol extract on *Shigella dysenteriae* 24162 ATCC was 250mg/ml and *Klebsiella pneumonia* 34089 ATCC 300mg/ml.

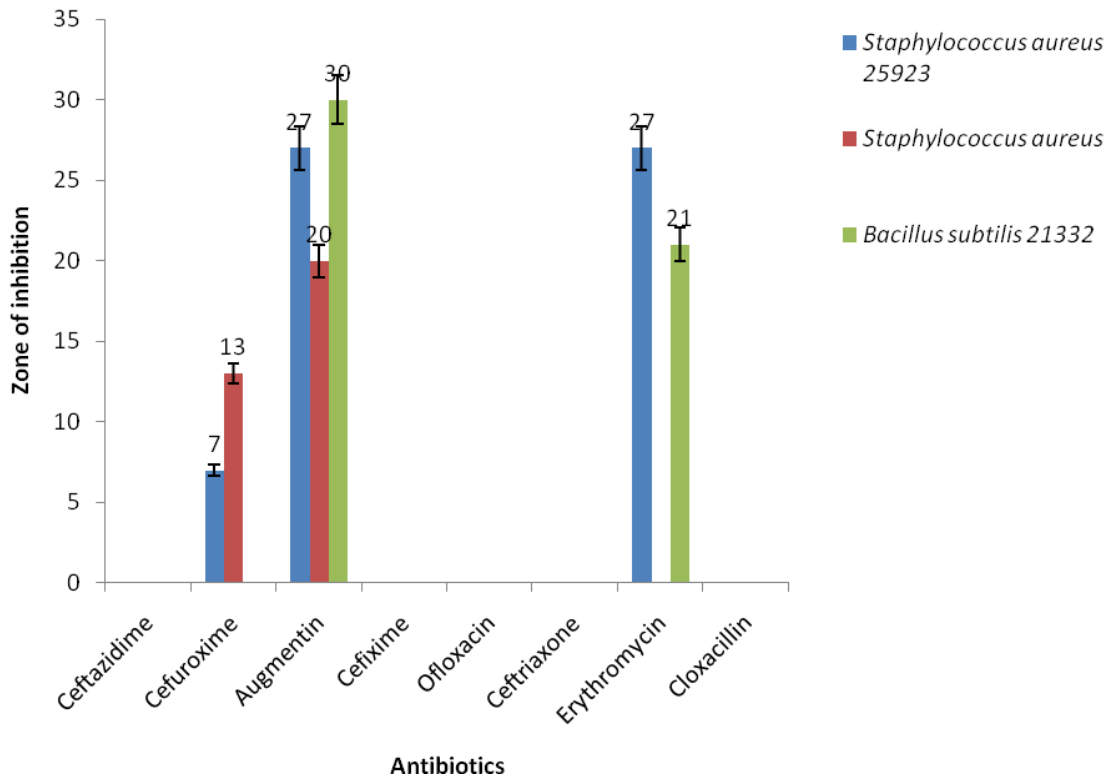


Figure 1: Antibiotic susceptibility pattern on Gram positive isolates

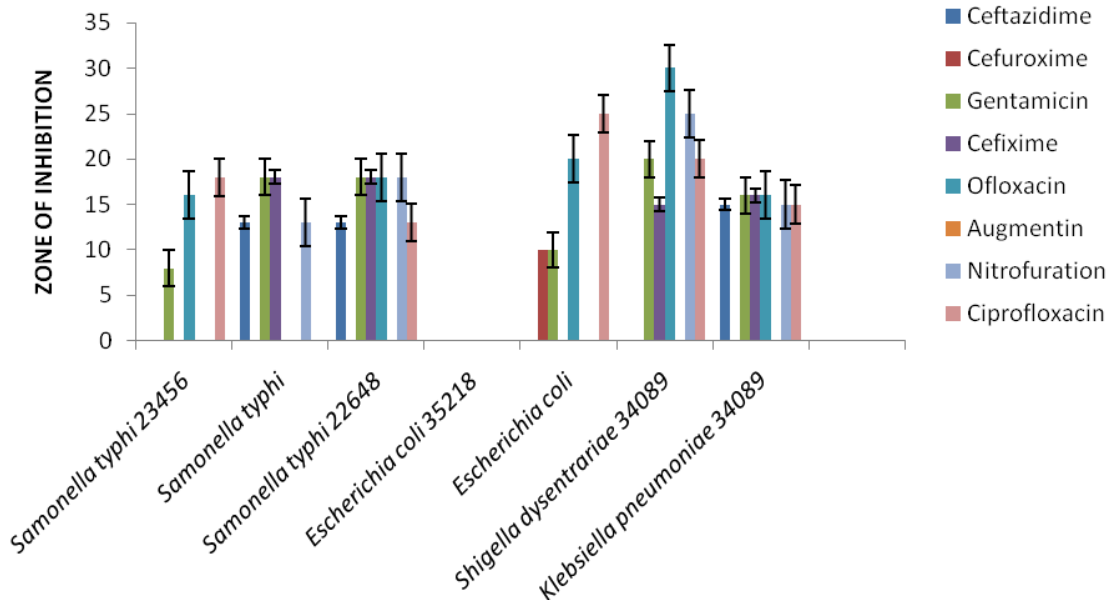


Figure 2: Antibiotic susceptibility pattern on Gram negative isolates

The MBC value for methanol and ethanol fruit extract on *Escherichia coli* 35218 ATCC and *E. coli*, *Staphylococcus aureus* 25923 ATCC and *S. aureus*, *Salmonella typhi* 22648 ATCC and *S. typhi* and *Bacillus subtilis* 21332 ATCC was 200 and 250mg/ml respectively, MBC value for methanol extract on *Shigella dysenteriae* 24162 ATCC was 300mg/ml and *Klebsiella pneumonia* 34089 ATCC 250 and 300mg/ml for methanol and ethanol extracts. The

results of the antimicrobial screening of the methanol, ethanol and acetone extracts of the peels and fruits against ten human pathogenic microbes, (bacteria) showed that methanol extract of the peel was more effective as compared to the methanol extract of the fruit and others. *Staphylococcus aureus*, *Escherichia coli* ATCC 35218, *Bacillus subtilis* ATCC 21332, *Staphylococcus aureus* ATCC 25923, *Samonella typhi* ATCC 22648, *Klebsiella pneumoniae* ATCC 34089, and *Shigella dysentrariae* ATCC 24162 among other microbes were comparatively more inhibited by both peel and fruit methanolic extracts (Tables 1a &1b). This result shows that the extract of *M. paradisiacal* peels has more antibacterial properties than the fruits. The ethanol extract of both fruit and peel exhibited higher antimicrobial activities at all concentrations compared to the acetone extract (Tables 1a &1b). The microbes against which the extracts were effective are pathogens already implicated in the etiologic and severity of human diseases. Thus, these plant extract may be useful in Pharmaceutical and medical formulations.

DISCUSSION

This study was designed to evaluate the antimicrobial activity of plantain (*Musa paradisiaca*) peel and fruit. Both the plantain peel and fruit extracts exhibited antibacterial potentials on Gram positive and Gram negative bacteria most especially with methanol extract. However, the bacterial species were more susceptible to plantain peel extracts than fruit extracts. Similar result was reported by [11]. Effects proving this might be the higher percentage of hydrocarbon, monoterpene and oxygenated monoterpene appreciated for their antibacterial potentials in the peel than fruit. It could also be noted that hence methanol extract exhibited higher antibacterial activity; it then signified that methanol has the potential of extracting the antibacterial substances from the plantain samples than other solvents. The antibacterial results obtained is similar to that reported by [12, 13]. Some literatures have reported information on the presence of bioactive molecules in many plants, which have served as food and medicine in health care man. Since the event of this scientific research on such discovery has been till date. The ideal about such research is to find lasting solutions to replacing synthetic antibiotics with naturally available phytochemicals present in plants for their low toxicity, low cost and readily available for human employment in disease treatment. [14] has reported ethanolic and aqueous extract of unripe *M. sapientum* fruit. In this study similar result was obtained with *M. parasidiaca* peel and fruit extracts. [15] has reported on the antibacterial activity of *M. sapientum* on some pathogenic bacteria.

Higher antibacterial effects than known synthetic antibiotics were exhibited on test bacterial species. The methanol extract of both peel and fruit had higher inhibition value on test bacteria than ethanol and acetone extracts. The microbes against which the extracts were effective are pathogens already implicated in the etiologic and severity of human diseases. Thus, the plant extract may be useful in antibacterial application. As a natural health product, *M. parasidiaca* preparations as food may be accepted more readily than prescription drugs for some patient groups, particularly in some communities afflicted with varying incidence of bacterial diseases and a paucity of culturally acceptable treatment options.

This result showed that *M. parasidiaca* though taken as food for carbohydrate source could serve as agent of bacterial inhibition.

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