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The Proximate Analysis, Mineral Composition, Phytochemical Screening and Antimicrobial Activity of Ripe and Unripe Peel Extract of *Musa Paradisiaca*

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ABSTRACT

Background/Objectives: *Musa paradisiaca* (Plantain) is used as a traditional therapeutic medicinal plant employed in various diseases.

This study was carried out to evaluate the nutraceutical potentials of the ripe and unripe peels of *Musa paradisiaca*. The phytochemical screening as well as antimicrobial activity of various extracts (methanol and ethanol) of ripe and unripe peels of *Musa paradisiaca* was also evaluated.

Methods: Proximate and mineral analyses of the samples were performed as per the standard methods of the Association of Official Analytical Chemists. Preliminary phytochemical screening of methanol and ethanol extracts of the peels was also carried out in accordance with standard methods. The antimicrobial activities of methanol and ethanol extracts of *M. paradisiaca* peel were tested *in-vitro* against isolates of clinical origin by agar well diffusion method.

Results: Phytochemical analysis of both peel extracts indicated the presence of flavonoids, terpenoids, starch, steroids, and reducing sugars. The mineral analysis indicated the presence of essential minerals such as iron, zinc, copper and manganese. The fat, protein, crude fibre and carbohydrate composition values of the unripe peel extract were found higher than the ripe samples. The ethanol and methanol extracts of both the ripe and unripe peels of *M. paradisiaca* used at various concentrations showed no antibacterial activities against the organisms.

Conclusion: This study concludes that ripe and unripe plantain fruit peels thought to be of little or no significance could serve as nutraceuticals and a medicinally vital material in animal health and probably humans.

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INTRODUCTION

Musa paradisiaca (common name: plantain) is an herbaceous, perennial, monocotyledonous plant belonging to the genus Musa along with (dessert) bananas (*M. sapientum*) in the family *Musacaceae*. (9, 37, 32, 31, 4, 28) While the plant is believed to be native to Southeast Asia, it is now cultivated extensively across both tropical and subtropical climates including Nigeria.(30, 38, 15, 34, 25) Plantain (*Musa paradisiaca*) is a

major food crop in Africa where its fruits are generally cooked or fried before consumption and serves as major sources of energy. (5) The fruit of *Musa paradisiaca* has been reported to be traditionally useful in treating diarrhoea, dysentery, intestinal lesions in ulcerative colitis, diabetes, uraemia, nephritis, gout, hypertension, cardiac disease etc. (17, 23) Plantain peels have been reported to be a good source of dietary fibres, carotenoids, polyphenols and other bioactive compounds which are beneficial to human health. (39, 9, 18) They have also been reported to be of substantial nutritional value. (3) Plantain peels have also been found useful as a promising raw material that could find useful industrial applications, especially in the agro-based and chemical industries. (27, 19) The antimicrobial activities of different parts including the peel of plantain plant useful for the treatment of a large number of human ailments have also been previously reported. (21, 12, 22, 5) There are limited reports on the nutraceutical potentials and chemical composition of plantain peels, and how they can be harnessed and bio-converted into useful materials. This study was carried out to evaluate the nutraceutical potential of ripe and unripe plantain peels by carrying out phytochemical screening as well as determining their proximate and mineral compositions. The antimicrobial activities of ripe and unripe Nigerian species of *Musa paradisiaca* peel extracts (ethanol and methanol) on selected human pathogens were also evaluated. The study was also carried out to promote the possible bioconversion of ripe and unripe plantain peels into useful products.

MATERIALS AND METHODS

Collection and preparation of samples

Healthy ripe and unripe plantain fruits were obtained from Okada market, Edo state, Nigeria. Fresh peels of ripe and unripe peels of *M. paradisiaca* were carefully separated and cut into smaller pieces and dried at room temperature after which they were grounded with a milling machine to get a powdered form of the peels. The powdered form of the peels was stored in separate labelled sterile air-tight containers. The grounded samples were used for phytochemical screening, antimicrobial susceptibility, mineral, and proximate composition analyses.

Extraction

50 g of both ripe and unripe grounded peels of *M. paradisiaca* were weighed differently and introduced into different clean jars. The methanol and ethanol extracts of each peel were prepared by soaking 50 g of each dried powdery sample in 500 mL of each solvent (methanol and ethanol) for 72 hours, during which the mixture was intermittently shaken. Filtration and concentration in a water bath (70°C) were subsequently carried out and resultant extracts were stored in clean, sterile, well labelled glass tubes and kept in a refrigerator (4°C) till further used.

Phytochemical screening

Screening for the presence of secondary metabolites such as saponins, flavonoids, glycosides, reducing sugars, alkaloids, phlobatannins, phenolic compounds, and terpenoids was performed as described by standard methods with slight modifications. (36, 33)

Proximate analysis

Proximate analysis of both ripe and unripe pulverized samples was carried out using the standard method of the Association of Official Analytical Chemists. (8) The parameters analysed were moisture, fat, ash, crude fibre, crude protein, and carbohydrate. Moisture was determined by the oven drying method in which samples were dried in a hot air oven at 105 °C until a constant weight was obtained, cooled in a desiccator, and reweighed. The difference in weight of the sample before and after drying was noted as moisture content. Ash content of the sample was obtained by properly ashing the samples in a muffle furnace at 550ºC for about 8 hr. Ash content of ash was calculated by subtracting the weight of ash from the initial sample weight. The percentage by mass protein content was analyzed by the Kjeldahl method in which samples went through the three essential steps of digestion, distillation, and titration. Fat content was determined by the semi-continuous solvent extraction method using the Soxhlet apparatus with n-hexane as the extraction solvent. The difference in weight of the sample after the extraction process using hexane was taken as the fat content of the sample. For crude fibre determination, the moisture-free and ether extracted sample was first digested with dilute sulphuric acid followed by dilute sodium hydroxide solution. The undigested residue collected after digestion was dried to constant weight in an oven at 100°C, cooled in a desiccator, and weighed. The weighed sample was then ignited in a muffle furnace at 550°C for 2h and loss in weight after ignition was registered as crude fibre content. Carbohydrate content was obtained based on the net difference between the total percentage composition and the sum of the percentage compositions of the other parameters determined for the proximate analysis of the samples.

Mineral analysis

The method used by Diane et al., 2002 was employed with slight modifications. (13) 5 g of the sample was weighed and put into the beaker and 10ml of the HCL and hydrogen peroxide (H_2O_2) were added heating vigorously for 30 minutes. After the heating mixture was filtered properly using filter paper. 5ml of the filtrate was taken and made up with distilled water inside a 50 mL volumetric flask. This was used for the digestion of the samples for the mineral analysis. The concentrations of the minerals in the digested sample were estimated with the Atomic

Absorption Spectrophotometer. Each analysis was carried out in triplicate

Microorganisms

Clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Streptococcus pyogenes* from different sources (Ear swabs, urine, semen, and wound) were obtained from the University of Benin Teaching Hospital (UBTH) Benin city, Edo state and Igbinedion University Teaching Hospital (IUTH), Okada, Edo state and used for the antimicrobial studies. All the isolates were maintained on nutrient agar slants at 4°C.

Preparation of Media, Extracts and Standards

All media were prepared according to the manufacturer's directions. For susceptibility testing, 1g of the various extracts were weighed and placed in respective universal bottles and properly labeled. About 2 mL of dimethylsulphoxide (DMSO) was added to each bottle to dissolve the extracts and made up with 8 mL of sterile distilled water to make a total volume of 100 mg/mL for each extract. A twofold serial dilution was carried out to obtain other concentrations. This was carried out for all the extracts. Preparation of the negative control was carried out by introducing 0.1 mL of DMSO was added to 9.9ml of sterile distilled water to make 1% dimethylsulphoxide (DMSO). The positive control was prepared by introducing 1 mL of gentamicin (80 mg/2mL) into 99 mL of sterile distilled water to make 400 µg/mL. Then 1 mL from the 400 µg/mL was collected and introduced into 39 mL of sterile distilled water to make a final concentration of 10 µg/mL.

Determination of Antibacterial Activity

An antibiotic susceptibility test was carried out using the disk diffusion method to determine the susceptibility of the clinical isolates to antibiotics. (11) A 10^{-2} dilution was done for all the clinical isolates to be used. A volume of 0.1 mL of the dilution of each isolate was introduced into separate Petri dishes containing set Muller Hinton media. Surface plating of the isolates was done using a sterile swab stick. With the aid of sterile forceps, the antibiotic discs containing a standard concentration of different antibiotics were applied to the surface of the various Petri dishes. Antibiotics present in the antibiotic disk include CAZ, Ceftazidime 30 mcg, CRX Cefuroxime 30mcg, GEN Gentamicin 10mcg CTR Ceftriaxone 30mcg ERY Erythromycin 5mcg CXC Cloxacillin 5mcg OFL Ofloxacin 5mcg AUG Augmentin 30mcg. The plates were incubated for 24hours at 37°C and the diameter of the zone of inhibition was measured with the aid of a calibrated rule and recorded. Antibiotic susceptibility results were interpreted using European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. (16) The agar diffusion technique was employed with slight modifications for susceptibility testing of isolates to methanol and ethanol extracts of *M. paradisiaca*. (2) A 10^{-2} dilution of the collected isolates were prepared for all isolates. 0.1ml of the dilution of each isolate was introduced into different prepared Muller Hinton Agar plates and with the aid of a sterile swab stick, surface plating of the isolates on the agar plates was carried out. Using a sterile cork borer (8mm diameter) holes for the different extract concentrations and controls (negative and positive) were bored onto the agar plates. Using a Pasteur pipette, 0.1 mL of the different concentrations and 0.1ml of the positive and negative controls were introduced into the respective holes bored for them. The experiment was carried out in triplicates. The plates were incubated for 24hours at 37°C and the diameter of the zone of inhibition was measured with the aid of a calibrated rule and recorded. This was carried out for the methanol and ethanol extracts of both the ripe and unripe extracts of *Musa paradisiaca*.

RESULTS

Phytochemical screening

Table 1 summarises the qualitative phytochemical analysis of *M. paradisiaca* peels. The results of the screening showed that both the ripe and unripe peels under investigation contained flavonoids, reducing sugars, terpenoid, starch and steroid. Saponin was detected only in the unripe peel.

Table 1. Phytochemical Screening of *Musa paradisiaca* peels

Phytochemical constituents	Ripe peels	Unripe peels
Flavonoids	++	++
Saponin	-	+
Cardiac Glycoside	+	-
Alkaloids	-	-
Phlobatannins	-	-
Phenolic compounds	-	-
Reducing sugar	++	++
Tannins (hydrolysable)	-	-
Tannins (condensed)	-	-
Terpenoids	+++	+++

Starch	++	++
Steroid	+	+

Mineral composition

Table 2 gives the mineral composition of the ripe and unripe *Musa paradisiaca* peels. Both peels showed the presence of four essential minerals at varying quantities while the quantities of four heavy metals detected were lower. One heavy metal (Cr) was not detected in both samples of the peels. Table 3 gives the proximate composition of the ripe and unripe peels. The various components detected were present in varying quantities in both the ripe and unripe *Musa paradisiaca* peels.

Table 2. Mineral composition of the *M. paradisiaca* peels

Minerals (ppm)	Ripe peel	Unripe peel
Fe	12.30+2.21	-0.73+0.08
Cu	1.92+11.60	14.73+8.67
Cd	0.023+0.001	0.024+.0.001
Mn	1.71+0.25	2.42+0.30
Ni	0.30+0.004	0.29+0.009
Co	0.07+0.22	0.46+0.09
Pb	0.30+0.16	0.28+0.09
Zn	0.66+0.00	0.38+0.00
Cr	0.00+0.00	0.00+0.00

Key: Fe = Iron, Cu = Copper, Cd = Cadmium, Mn = Manganese, Ni = Nickel, Co = Cobalt, Pb = Lead, Zn = Zinc, Cr = Chromium

Table 3 Proximate composition of the ripe and unripe *Musa paradisiaca* peels

Component	% composition Ripe peels	% composition Unripe peels
Crude protein	4.38	6.13
Ash	13.67	12.04
Fibre	8.78	12.58
Fat	11.74	13.14
Moisture	23.26	16.41
Carbohydrate	38.17	39.70

Antimicrobial susceptibility test

Supplementary table 1 gives the antibiotic susceptibility test results of the isolates used in this study. Two isolates *Escherichia coli* and Coagulase negative *Staphylococcus aureus* showed 100% resistance to the antibiotics. One of the *Staphylococcus aureus* isolates was sensitive to cefuroxime. The *Klebsiella spp* isolate was sensitive to ceftazidime, cefuroxime, gentamicin, ceftriaxone and ofloxacin. Significantly the extracts had no antimicrobial activity on the isolates tested. Only the positive control 10 µg/mL gentamicin had activity on some of the isolates. Dimethylsulphoxide (DMSO) also had no activity on the isolates.

DISCUSSION

Phytochemicals are natural bioactive compounds present in plants and are useful as a result of their protective and disease preventive properties hence their medicinal and pharmacological importance. (29) The result from this study slightly compares with a previous study carried out by Ighodaro, 2012 who recorded flavonoids, glycosides and reducing sugar, alkaloids, and phlobotannins to be present in both ripe and unripe plantain peels. (21) Another study by Ibhafidon et al., 2020 recorded flavonoids, saponins, glycosides and reducing sugar, alkaloids, and phenolic compounds to be present in both ripe and unripe peels of *Musa paradisiaca*.(20) These previous results confirm the result obtained in this study.

These phytochemicals have been shown to have multiple medicinal and pharmacological effects (antibacterial, antihypertensive, antioxidant, free radical scavenging abilities, anti-carcinogenic, anti-diabetic, and anti-inflammatory activities). (35, 21, 7, 26, 20) Significantly the extracts had no antimicrobial activity on the isolates tested compared to the positive control 10 µg/mL gentamicin which had activity on some of the isolates. This contrasts with previous reports that show the antimicrobial activity of ripe and unripe peels of *Musa paradisiaca*. (21, 5) The absence of antimicrobial activity of the extracts in this study could possibly be a result of the location and environmental factors of where the plant (*Musa paradisiaca*) was collected. Another study carried out by Ayuba et al. 2016, showed that ethanol extracts at 25, 50, 75 and 100 mg/mL had no activity against *Staphylococcus aureus*, *Klebsiella pneumonia*e while only the concentration of 100 mg/mL had little activity on *Escherichia coli.* (10) This slightly correlates with this study as the ethanol extracts of both ripe and unripe peels showed no activity against the selected isolates. The absence of microbial activity of the various extract on the isolates may also be possible due to the concentration of extract used. Higher concentrations of the extracts may be required to possibly have inhibitory effects on the isolates as the phytochemical screening show that both the ripe and unripe peel samples contain active phytochemical components. Another possible factor could be the limited number of isolates tested. Testing of a large number of samples could possibly reveal some isolates to be sensitive to the extracts. The resistance potential of the isolates as seen from the antibiotic susceptibility results could be another possible factor.

The proximate composition of both ripe and unripe peels in this study was in varying quantities. Proximate composition results from this study carried out correlate but with slight variations with the study carried out by Ibhafidon, 2020, where the composition of the carbohydrate, fibre, fat, and ash was higher in the unripe samples compared to the ripe samples. (20) In another previous study by Ighodaro, 2012, the percentage compositions for carbohydrate, fibre and moisture content were higher in the unripe samples compared to the ripe samples. (21) Determination of the ash component of a sample gives an index of the amount of minerals present. (1) Moisture content is a very important parameter in food analysis as it determines the means of processing as well as the shelf-life and storage of the food. (6) The protein contents of both samples can possibly be an important source contributing significantly to the daily amount required by humans. Fat and carbohydrate are good sources of energy for animals and humans. (1) Previously, the crude fibre content in diets has been shown to aid digestive processes thereby promoting livestock and human health. (1)

The mineral compositions of both the ripe and unripe peels were in varying quantities. Both ripe and unripe plantain peels are good sources of essential minerals; iron, copper, manganese, and zinc as seen in this study. The levels of Manganese (Mg), and Zinc (Zn) were quite low in this study compared with a previous study by Ighodaro, 2012 where Iron (Fe), Zinc (Zn), Copper (Cu), Manganese (Mn) were recorded in significantly higher percentages. (21) Previous studies have reported these minerals to have therapeutic importance required for normal growth, development, and proper functioning of the body. (14, 24) The absence of Chromium (Cr) which is a heavy metal and the low quantity in which Cadmium (Cd), Nickel (Ni) and Lead (Pb) which are also heavy metals is an important aspect in the light of the toxicity associated with heavy metal accumulation in the body. Peels of plantain fruit have been proven to have a high tendency to absorb heavy metals even when present in the smallest concentrations. (19)

CONCLUSION AND RECOMMENDATIONS

Results from this study confirm that unripe and ripe plantain peels are good sources of nutrients, minerals and phytochemicals. They could be nutritionally studied and properly processed as good sources of nutrients. The presence of phytochemicals also shows its potential to serve as nutraceuticals and a medicinally vital material in animal health and probably humans.

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Antibiotics➡ Organisms ⬇	**Source**	**CAZ**	**CRX**	**GEN**	**CTR**	**ERY**	**CXC**	**OFL**	**AUG**
Klebsiella spp	Urine	S	S	S	S	R	R	S	R
Escherichia coli	Urine	R	R	R	S	R	R	R	R
Streptococcus pyogenes 3	Semen	R	S	R	S	R	R	NB	R
Staphylococcus aureus 4	Semen	R	R	R	R	R	R	NB	R
CON *Staphylococcus aureus 14*	Wound isolate	R	R	R	R	R	R	R	R

Supplementary table 1 Antibiotic susceptibility test results

Key:

CAZ = Ceftazidime 30mcg, CRX = Cefuroxime 30mc, GEN = Gentamicin 10mcg, CTR = Ceftriaxone 30mcg, ERY = Erythromycin 5mcg, CXC = Cloxacillin 5mcg, OFL = Ofloxacin 5mcg, AUG = Augmentin 30mcg, R= Resistance, S= Sensitive, NB = No breakpoint in EUCAST table