



Comparative Evaluation of the Proximate Composition and Antibacterial Activity of Ground *Musa paradisiaca* (Plantain) Peels and Leaves

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Authors' contributions

This work was carried out in collaboration between all authors. Author ACCE designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors OMO and KLA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study was conducted between May and August, 2015. It determined and compared the proximate composition (measured in percentage, %) of the ground *Musa paradisiaca* peels and leaves, and the antibacterial activity (measured in millimeter, mm) of the aqueous and ethanol extracts (at concentration of 100 mg/ml) of the respective sample, using standard protocols. The peels percentage yield (91.59±1.26%) was higher (p<0.05) than that of the leaves (84.29±1.54%). Apart from the protein content (18.09±0.09%), the other proximate parameters viz: fat (9.60±0.16), crude fibre (39.17±0.83) and ash (22.24±0.23) in the ground leaves were higher (p<0.05) than the corresponding value in the peels. Higher carbohydrate (32.47±0.48) and moisture (12.78±0.58) were recorded in the peels than in the leaves. The anti-bacterial activity (mm) of the aqueous and ethanol extracts of the leaves against *Staphylococcus aureus* was 9.33±0.58 and 12.33±1.15, respectively while against *Escherichia coli* was 14.00±1.73 and 18.67±1.15, respectively. The ethanol extracts of the peels had higher (p<0.05) antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (14.33±1.53, 15.00±2.00) respectively than the respective value obtained for aqueous extracts. However, unlike the activity against *S. aureus*, there was no

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difference in activity against *E. coli* based neither on the extracting solvents nor on the study samples. Thus, *Musa paradisiaca* peels and leaves could serve as nutrient and antibacterial sources. However, the peels compared to the leaves may serve as better source for carbohydrate but with poor storability while the leaves may serve as better source for the other determined nutrients and as antibacterial against the studied pathogens. The study further revealed that the activity of the samples against *E. coli* was unaffected by the solvent type and that ethanol could be preferred to water as the extracting solvent for activity against *S. aureus*. Further studies are required, hence recommended to harness the present findings and ultimately to reduce their solid waste contribution.

Keywords: *Escherichia coli*; *Staphylococcus aureus*; aqueous; ethanol; percentage yield.

1. INTRODUCTION

Generally, proximate content and antibacterial activity determination of plants and plant parts provide basic information for discovering and developing new sources for foods and drugs. The need to discover and develop new foods and drugs sources was necessitated by the increasing human health challenges and food shortages [1], adverse effects of apparently harmless foods including newly developed foods, and food condiments [2,3] that ultimately overwhelmed the existing foods and drugs. This is compounded by the diminishing antibacterial efficacy against bacteria-induced diseases owing to increasing drug and even multiple drug resistance by disease causing bacteria [4,5].

Musa paradisiaca is a mono herbaceous perennial crop that is in the family of *Musaceae*, genus *Musa* and specie *paradisiaca* [6]. It is a tropical crop and is an important staple food in Africa [7] including Nigeria [8]. The plant is tall with a sturdy pseudo stem that has large broad leaves arranged spirally at the top. The leaves have large blades with a pronounced central midrib and obvious veins. Each pseudo stem produces a group of flowers from which the fruits develop in a hanging cluster. *Musa paradisiaca* is also known as 'ogede', 'agbagba' and 'apanda' in Yoruba, and as 'ayaba' in Hausa [9]. In Igbo language, the various names are used to differentiate plantain as a whole plant and as the unripe or ripe fruits. For instance, in Ojoto and environs, the whole plant is known as 'ojioko' and the unripe fruit is known as 'jioko' while the ripe fruit is known as 'ogade'/'ogede'. Literally, 'jioko' means 'yam pod' or 'yam pod in a bunch' probably because plantain and yam are essentially consumed similarly boiled, roasted, pounded and fried.

Plantain fruits are available in Nigeria throughout the season and are employed in the folklore

management of diseases such as ulcer, wound healing and many others due to its anti-ulcerogenic, antimicrobial and analgesic properties [10]. This could be so since fruits generally contain bioactive compounds. For instance, *Citrus limonum* (lemon) contain esculetin reported to improve markers of health functions in rats [11]. Many recipes are made from plantain fruits. For instance, the unripe fruits are processed into flour and thickened into paste by stirring in boiling water. The ripe or unripe mature fruits are consumed boiled, steamed, pounded, roasted or fried into chips while the overripe plantains are fried with palm oil [8]. Thus, the demand for plantain fruit in Nigeria is high. This could result to abundant waste generation in the form of plantain peels that are essentially discarded as waste, thereby constituting a menace to the environment. In addition, plantain leaves are spatially used but not as food, hence are essentially discarded on harvesting the plantain fruits.

Plantain is rich in phyto-nutrients hence has nutritional value [12,13]. The peels could be good source of bioactive compounds but as major waste products of various fruits are essentially discarded. Plantain leaves may have nutritional and medicinal properties but were in most cases utilized for neither purpose. Generally, nutrient content and antibacterial activity determination are basic steps to developing novel nutraceuticals, warranting this study designed to determine and compare the proximate and antibacterial properties of plantain (*Musa paradisiaca*) peels and leaves. The possible contribution of these samples, notably the peels, to food supply could be high [14]. The study objectives include the determination of proximate contents viz: moisture, ash, crude fibre, fat, protein and carbohydrate contents in the peels and leaves flour and the determination of the anti-bacterial activity of the aqueous and ethanol extracts of the peels and leaves against two

bacteria pathogens (*Escherichia coli* and *Staphylococcus aureus*). In particular, the determination of the proximate content of the peels could justify its comparison with that of earlier study [14] and in this study with that of the leaves.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

The solvent, ethanol and other chemicals used, including those used in the preparation of reagents, were of analytical grade and product of British Drug House Laboratory, England. This study was conducted between May and August, 2015 at the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria.

2.2 Collection and Identification of Plant Materials

A mature but unripe plantain bunch was purchased (and harvested, with the epicarp/peel still green, along with the green leaves) at Ndioro, a town near Michael Okpara University of Agriculture, Umudike Nigeria. It was identified as giant/elephant plantain specie of *Musa paradisiaca* by Dr. Garuba Omosun of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Nigeria. The whole of the leaves, including the mid rib, was used in the study.

2.3 Samples Preparation

The plantain fruits were thoroughly washed with clean water. The peels were removed and chopped into bits using a home choice European knife. The leaves were separately chopped into bits. The samples (peels and leaves) were separately placed on a foil and weighed with a Satorious Digital Weighing Balance, Model BP210S, Germany before and after sun drying for four days to obtain the wet weight and dry weight respectively. The respective dry weight sample was separately ground into powder using Arthur Thomas Laboratory Mill Crypto model, USA, covered separately in a labeled white nylon and kept in the desiccator pending use. There was no sampling plan for the choice of the samples used in this study. The samples were selected based on their waste status as adjudged from their underutilization or non use either in diet or drug.

2.4 Determination of Percentage Yield of the Sample

The respective sample percentage yield was calculated from the sample wet weight (g) and the dry weight (g) thus:

$$\text{Yield (\%)} = \frac{\text{Sample dry weight}}{\text{sample wet weight}} \times 100$$

2.5 Sample Extraction

The aqueous and ethanol extracts respectively of the *Musa paradisiaca* peels and leaves were separately obtained as described previously [15]. However, 10 g of the respective ground sample, weighed using Sartorius Digital Weighing Balance, was immersed in 100 ml instead of 200 ml of the respective extraction solvent (ethanol or water) contained in a conical flask. The solution was shaken intermittently for 24 hours and thereafter filtered using Whatman no.1 filter paper into a clean beaker. The respective solvent was recovered from the filtrate using a Soxhlet apparatus and the filtrate finally evaporated to dryness using a steam bath set at 100°C.

2.6 Proximate Determinations

2.6.1 Ash content

The ash content was determined by the furnace incineration gravimetric method [16]. A measured weight (5 g) of each sample was placed on a previously weighed Porcelain crucible and placed on a muffle furnace at 550°C. The sample was allowed to burn to grey ash before the crucible was carefully removed from the furnace, cooled using a desiccator and re-weighed. The difference in ash weight was obtained and expressed as percentage of the sample weight.

2.6.2 Moisture content

The moisture content of each sample was determined by the gravimetric method [17,18]. A measured weight of the fresh samples (5 g) was placed in a previously weighed moisture can and dried for three hours on an oven set at 105°C, cooled in a desiccator and reweighed. This process was repeated at intervals until a constant weight was obtained. The moisture lost was determined and expressed as a percentage of the sample weight.

2.6.3 Protein content

The protein content of the sample was determined by the Kjeldahl method [19]. 0.5 g of

each sample was mixed with 10 ml of concentrated sulphuric acid (H_2SO_4) in a Kjeldahl digestion flask. A tablet of selenium catalyst was added to it and the mixture was digested by heating under a fume cupboard until a clear solution was obtained. Each of the digest was carefully transferred into a 100 ml volumetric flask and made up to the mark with distilled water. A 10 ml portion of each digest was mixed with an equal volume of 45% NaOH solution in a Kjeldahl distilling unit. The mixture was distilled and the distillate collected into 10 ml of 4% butyric acid solution containing three (3) drops of mixed indicator, bromocressol green and methyl red. 50 ml of the distillate was collected and titrated against 0.02 N H_2SO_4 solution until it changed from green to a deep red end point. A reagent blank was also digested, distilled and titrated, just as the sample. The total nitrogen thus determined was multiplied by the factor 6.38 to obtain the protein content.

2.6.4 Fat content

The fat content was determined by the continuous solvent extraction method using a Soxhlet extractor [17,18]. Five gram (5 g) of each sample was wrapped with a pre-weighed Whatman filter paper No 40. The wrapped sample was placed in a Soxhlet column flask mounted onto a weighed oil extraction flask containing about 300 ml of petroleum ether (40-60°C boiling point). The wrapped sample was defatted twice and the fat content determined by weight difference of each sample and expressed as a percentage of each sample weight.

2.6.5 Carbohydrate content

The carbohydrate content of the test samples was estimated from the arithmetic difference [17,18]. The carbohydrate content was calculated and expressed as the nitrogen free extract (NFE) as shown below:

$$\% \text{ CHO (nitrogen free extract)} = 100 - \% (a+b+c+d+f)$$

Where: *a* = protein, *b*=fat, *c*=ash, *d* = fibre and *f* = moisture.

2.6.6 Crude fiber

This was determined by the method of James [18]. Five gram (5 g) of each sample was defatted (as in fat determination) and the

defatted sample boiled in 200 ml of 1.25% H_2SO_4 solution under reflux for thirty minutes. After that, the sample was washed with enough hot water using a twofold muslin cloth to trap the particles. The washed sample was carefully transferred into a weighed porcelain crucible and dried for an hour on an oven set at 105°C, cooled in a desiccator and reweighed. The loss in weight after drying was calculated as the crude fibre content and expressed as a percentage of the sample weight.

2.7 Tested Bacterial Strains

The bacterial strains used for the antibacterial test, *Staphylococcus aureus* and *Escherichia coli* were clinical isolates provided by the Central Laboratory of National Root Crop Research Institute Umudike Abia state, Nigeria. Generally, the choice of *E. coli* and *S. aureus* in the study of anti-bacterial activity was that they respectively represent common gram negative and gram positive bacterial pathogens.

2.8 Antibacterial Activity Test

The disc agar diffusion method was used to determine the antibacterial activity of the extracts as reported earlier [20]. Incubation was at 37°C for 24 hours under aerobic condition. The antibacterial activity was determined by the Kirby-Bauer method that involved measuring the diameter (in millimetres) of the zone of inhibition formed around the discs. The antibacterial activity test was performed in triplicate and the mean zone of inhibition calculated.

2.9 Statistical Analysis

The data obtained by triplicate determinations were subjected to analysis of variance (ANOVA) using SPSS 16.0 for Windows. Difference at a *p* value < 0.05 was regarded as statistically significant. Results were expressed as mean± standard deviation (SD).

3. RESULTS AND DISCUSSION

From Table 1, the percentage yield of the peels sample (91.59±1.26%) was higher (*p*<0.05) than that of the leaves (84.29±1.54%).

As shown on Table 2, apart from protein content (18.09±0.09%), the fat, crude fibre and ash value (%) respectively in the leaves flour (9.60±0.16, 39.17±0.83, 22.24±0.23) was higher (*p*<0.05) than the corresponding value in the peels. Higher

carbohydrate (32.48±0.48) and moisture (12.78±0.58) contents were recorded in the peels than in the leaves.

As shown on Table 3, the anti-bacterial activity (mm) of the aqueous and ethanol extracts of the leaves against *Staphylococcus aureus* was 9.33±0.58 and 12.33±1.15, respectively while against *Escherichia coli* was 14.00±1.73 and 18.67±1.15, respectively. The ethanol extracts of the peels had higher ($p < 0.05$) antibacterial activity against *Staphylococcus aureus* and

Escherichia coli (14.33±1.53, 15.00±2.00) respectively than the respective value obtained for aqueous extracts. However, unlike the activity against *S. aureus*, there was no difference in activity against *E. coli* based neither on the extracting solvents nor on the study samples.

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Table 1. Percentage yield of the *Musa paradisiaca* (plantain) leaves and peels samples

Samples	Initial or wet weight (g)	Final or dry weight (g)	Yield (%)
Leaves	320.00±2.13	269.74±2.21	84.29±1.54
Peels	273.00±1.33	250.05±1.12	91.59±1.26
Difference	±47.00*	±19.69*	±7.30*

Result = Mean ± SD of triplicate determinations. * = Difference is significant ($p < 0.05$), ns = Difference is not significant ($p > 0.05$)

Table 2. The proximate content (%) of the *Musa paradisiaca* (plantain) peels and leaves

Parameters	Peels (%)	Leaves (%)	Difference (%)
Ash	17.84±0.24	22.24±0.23	±4.40*
Fibre	15.98±0.16	39.17±0.83	±23.19*
Fat	3.81±0.16	9.60±0.16	±5.79*
Protein	17.12±0.10	18.09±0.09	±0.97 ^{ns}
Moisture	12.78±0.58	8.99±0.16	±3.79*
Carbohydrate	32.47±0.48	1.91±0.49	±30.56*

Result = Mean ± SD of triplicate determinations. * = Difference is significant ($p < 0.05$), ns = Difference is not significant ($p > 0.05$)

Table 3. Anti-bacterial activity (inhibition zone diameter, IZD (mm) at a concentration of 100 mg/ml of the ethanol and water extracts of *Musa paradisiaca* (plantain) peels and leaves

According to extracting solvents	Bacteria species	According to study samples		Difference (mm) between the peels and leaves extracts	Difference (mm) between the extracting solvents
		Plantain peels	Plantain leaves		
Ethanol extract (100mg/ml)	<i>E. coli</i> (IZD, mm) (gram -ve)	15.00±2.00	18.67±1.15	±3.67*	
Water extract (100mg/ml)	<i>E. coli</i> (IZD, mm) (gram -ve)	10.33±0.58	14.00±1.73	±3.67*	
	Difference between the activity of the extracting solvents against <i>E. coli</i> (mm)	±4.67*	±4.67*	±0.00 ^{ns}	±0.00 ^{ns}
Ethanol extract (100mg/ml)	<i>S. aureus</i> (IZD, mm) (gram +ve)	14.33±1.53	12.33±1.15	±2.00*	
Water extract (100mg/ml)	<i>S. aureus</i> (IZD, mm) (gram +ve)	0.00±0.00	9.33±0.58	±9.33*	
	Difference in activity between the extracting solvents against <i>S. aureus</i> (mm)	±14.33*	±3.00*	±7.33*	±11.33*

Result = Mean ± SD of triplicate determinations. * = Difference is significant ($p < 0.05$), ns = Difference is not significant ($p > 0.05$)

sources was necessitated by the increasing human health challenges and food shortages [1], warranting this study aimed at determining and comparing the proximate and antibacterial properties of *Musa paradisiaca* peels and leaves. The peels percentage yield ($91.59 \pm 1.26\%$) which was higher ($p < 0.05$) than that of the leaves ($84.29 \pm 1.54\%$) could be a pointer to lower moisture loss from the peels than from the leaves [21] and possibly lower stability of the peels than that of the leaves [20]. This appears to be supported by the higher moisture content recorded in the peels than in the leaves (Table 2). The moisture content in the leaves compared with, while that in the peels was higher than, that recorded for plantain bract [8]. Apart from protein content, the other proximate contents viz: fat, crude fibre and ash in the leaves flour were higher ($p < 0.05$) than the corresponding value in the peels (Table 2). This could suggest higher dietary mineral, protein, fat, fibre [22,21] and probably higher attendant health benefits in the leaves than in the peels samples. Protein, for instance, is needed for healthy state in animal [23], and high ash content generally indicates high mineral content.

The fat content in the peels compared with, while that in the leaves was higher than, $2.5 \pm 0.2\%$ in asparagus bean [24,25], indicating that the plantain leaves could be a rich fat source. Crude fat content in the leaves and peels were higher than that recorded for plantain bract [8]. The ash content of the samples was comparatively higher than those reported for agricultural hulls [26], plantain bract [8] and the range (6.70-8.00%) reported by Osabor et al. [27]. However, the ash content in either sample compared with the range (17.59-22.30%) reported for ripe and unripe plantain peels [14]. Crude protein content in either the leaves or peels was higher than that recorded for plantain bract [8]. Similarly, the protein content in either the peels or leaves was higher than the range (5.6-6.62%) reported by Osabor et al. [27]. Crude fibre measures the lignin content of food that comprises phenolic acids and hemicellulose polymers [28]. Crude fibre content in the leaves and peels were higher than that recorded for plantain bract [8]. The fibre content in the peels and leaves was higher than that in sweet orange leaves [21], in asparagus bean ($5.72 \pm 0.14\%$) [24,25], in African breadfruit (1.55%) and soybean (6.46%) [22], in watermelon rind and leaves [29] and in the plantain bract and in African star apple (*Chrysophyllum albidum*) fruit pulp [30]. The higher fibre in the leaves is significant and

suggests that the plantain leaves could serve as significant source for dietary fibre. High fibre content could increase stool bulk, decrease the gastro-intestinal tract motility, reduce cholesterol level and enhance the removal of carcinogens, potential mutagens, steroids, bile acids and xenobiotics [31]. Thus, the nutritional importance of dietary fibre and the possible abundance in plantain leaves warrant further studies to harness the utilization of plantain leaves-sourced dietary fibre.

Higher carbohydrate content in the peels ($32.47 \pm 0.48\%$) than in the leaves could be an indication that the plantain peels may supply more carbohydrate related energy in animals when consumed. The carbohydrate content in the peels compared with that reported by Ighodaro [14] for ripe (42.95%) and unripe (48.18%) plantain peels but lower than that recorded for plantain bract [8]. The higher moisture content in the peels ($12.78 \pm 0.58\%$) than in the leaves supported the higher percentage yield in the peels as recorded in this study and the suggestion thereto. The moisture in the peels and leaves (Table 2) was lower than that reported by Osabor et al. [14] in the leaves (40.3%) and roots (29.2%) of miracle fruit (*Synsepalum dulcificum*) and that reported by Edem and Dosunmu [32] in *Chrysophyllum africanum* ($66.67 \pm 0.02\%$). However, the moisture in the leaves compared with the range (3.81-9.69%) reported earlier [22,29,21]. The lower anti-bacterial activity (mm) of the aqueous than the ethanol extracts of the leaves against the tested pathogens (*Staphylococcus aureus* and *Escherichia coli*) was a pointer that the aqueous extract of the leaves may not be as effective as that of the peels against the tested pathogens. Further to this, the aqueous extract of the plantain peels had no activity against *S. aureus*, implying that the water extract of plantain peels is not bacteriostatic against *S. aureus* hence could not be useful as broad spectrum antibacterial [33,34,35]. Compared to the aqueous extract, the higher ($p < 0.05$) activity of the ethanol extracts of the peels against *Staphylococcus aureus* and *Escherichia coli* (14.33 ± 1.53 , 15.00 ± 2.00 , respectively) could be due to the fact that ethanol being more volatile dissolved more phytochemicals with anti-microbial properties than water [1]. *Musa paradisiaca* peels and leaves extracts inhibited growth of *Escherichia coli* and *Staphylococcus aureus* except for the aqueous peel extract which showed no inhibition for *Staphylococcus aureus*. Generally, anti-bacterial activity of plant origin is not associated

with any side effects, nevertheless, toxicological evaluation of these samples extracts need to be carried out for clarification, hence are warranted. However, unlike the activity against *S. aureus*, there was no difference in activity against *E. coli* based neither on the extracting solvents nor on the study samples. This could suggest that the activity of the samples against *E. coli* was unaffected by the solvent type even at very high sample concentration. Thus, ethanol could be preferred to water as the extracting solvent for activity against *S. aureus*. The high concentration of the extracts (100 mg/ml) as used in this study, though expected to elicit measurable activity from the crude extracts at minimal test and cost, was a notable limitation of the study, warranting the use of lower concentration of the crude extracts in further and similar studies.

4. CONCLUSION

Thus, *Musa paradisiaca* peels and leaves could serve as nutrient and antibacterial sources. However, based on the proximate mix result, the peels as compared to the leaves may serve as a better source for carbohydrate but with poor storability while the leaves may serve as better source for the other determined nutrients and as antibacterial against the studied pathogens. The study further revealed that the activity of the samples against *E. coli* was unaffected by the solvent type and that ethanol could be preferred to water as the extracting solvent for activity against *S. aureus*. Further studies are required, hence recommended to harness the results of this study and ultimately to reduce their solid waste contribution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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