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Comparative Proximate and Antibacterial Properties of Milled *Carica papaya* (Pawpaw) Peels and Seeds

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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 EMH and IAO 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, conducted at the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria between May and August, 2015, determined and compared the proximate (percentage, %) and antibacterial (millimeter, mm) properties of milled *Carica papaya* Linn peels and seeds, using standard protocols. Each sample extract (100 mg/ml) was tested against three bacteria - *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus)* and *Pseudomonas aeruginosa (P. aeruginosa)*. The percentage yield of the peels (61.29 %) was lower ($p<0.05$) than that of the seeds (74.34 %). The ash (14.07±0.06) and carbohydrate (53.22±0.05) in the peels was respectively higher ($p<0.05$) than that in the seeds (10.51±0.02, 22.37±0.03) while the fibre (24.09±0.07), fat (0.99±0.08), protein (4.21±0.02) and moisture (3.42±0.03) in the peels was lower ($p<0.05$) than the corresponding content in the seeds (26.31±0.02, 29.62±0.02, 7.41±0.01 and 3.78±0.04). Beside *P. aeruginosa* (that was not susceptible to the aqueous extract of the seeds), the activity of the aqueous extract of the peels against *E. coli* and *S. aureus* was lower ($p<0.05$) than that of the seeds. The activity of the ethanol extract of the peels against *E. coli* (17.33±1.15), *S. aureus* (15.00±1.73) and *P. aeruginosa* (14.33±1.15) was in each case higher ($p<0.05$) than that of

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the seeds (14.33±0.58, 0.00±0.00, 9.67±1.15). The aqueous and ethanol extracts of the seeds respectively had no activity against *P. aeruginosa* and *S. aureus*. Thus, the pawpaw peels could be a durable source for mineral and carbohydrate with broader activity and higher potency against the tested bacteria while the seeds could be a source for protein, dietary fibre and fat, but with limited durability and activity against the tested bacteria. Harnessing the results of this study may enhance the use of these samples in diets and drugs to ultimately reduce their environmental burden.

Keywords: Escherichia coli; Staphylococcus aureus; Pseudomonas aeruginosa; aqueous; ethanol.

1. INTRODUCTION

Human health challenges are on the increase and could be compounded by adverse effects of apparently harmless foods and food condiments [1,2] thereby overwhelming the existing drugs. As a consequence and coupled to food shortage, there is increasing search for scientific basis to use plant and plant parts as foods and drugs, hence the need to shift such search to food and fruit wastes. Furthermore, harnessing the use of food and fruit wastes as diets and drugs could ultimately reduce environmental waste burden and the attendant public health implications. *Carica papaya* which belongs to the family Caricaceae, is found in tropical regions of Africa, Central and South America [3]. *Carica papaya* plant is short-lived, evergreen and grows to the height of 3 – 10 m [4] and even up to 20 - 30 m [5]. Its hollow trunk, marked with leaf scars, rarely branches [5].

In particular, the pawpaw fruit is known as '*okwuru ezi*' in Ojoto and neighbouring towns in the south eastern Nigeria. The fruit is oval in shape with greenish thin skin that ripens to yellowish colour. The thin skin covers the orange to orange-red edible flesh or pulp which surrounds a central cavity filled with small black seeds [5]. Generally fruits contain bioactive compounds. For instance, *Citrus limonum* (lemon) contain esculetin reported to improve markers of health functions in rats [6]. Similarly, the pawpaw fruit contains bioactive components, including phytoalexin, dietary minerals and fibre [3-5]. Crude *papaya* seed extract had antiprotozoal activity [7] while the peels had antifungal activity [3,5].

The pawpaw plant is regarded as nutraceutical fruit plant [4] owing to the numerous nutritional and health benefits of the various parts [3,4,8]. Thus, there is high consumption of pawpaw fruits resulting in the generation of wastes in the form of peels and seeds. These warranted this study aimed at determining and comparing the proximate and antibacterial properties of pawpaw peels and seeds. In line with achieving the above aim, the proximate content (fat/oil, fibre, moisture, carbohydrate, protein and ash) in the samples flour and the activity of the aqueous and ethanol extracts of the samples against three bacteria pathogens, *E. coli*, *S. aureus* and *P. aeruginosa* were determined and compared. The result of this study may provide scientific basis for, and insight to harness, the nutraceutical uses of pawpaw fruit peels and seeds.

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 products of reputable companies, including Sigma Chemical Company, St. Louis, U.S.A. and British Drug House (BDH) Ltd., Poole, 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

The pawpaw fruits were purchased from Ndioru market in Ikwuano Local Government Area, Abia State, Nigeria. The fruit sample was identified by Mr. Obi, a taxonomist in the Central Laboratory of National Research Root Crop Institute Umudike, Nigeria as *Carica papaya* Linn ("Agric pawpaw or oblong shaped variety).

2.3 Samples Preparation

The pawpaw fruits were thoroughly washed with clean water, sliced longitudinally into four equal parts and peeled, using a home choice European knife. The seeds were picked from the pulp and washed with clean water. The peels were chopped into bits. The samples (peels and seeds) 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 milled using Arthur Thomas Laboratory Mill Crypto model, USA, covered separately in a labeled white nylon and kept in the desiccator until used.

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:

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

2.5 Sample Extraction

The aqueous and ethanol extracts respectively of the samples (peels and seeds) were separately obtained as described previously [9]. However, 10 g of the respective milled sample was immersed in 100 ml instead of 200 ml of the respective extraction solvent.

2.6 Proximate Determination of the Samples Flour

2.6.1 Ash content

The ash content was determined by the furnace incineration gravimetric method [10]. A measured weight (5 g) of each sample was placed on a previously weighed Porcelain crucible and placed on a muffle furnace at 550℃. 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 [11,12]. A measured weight of the fresh samples (five grams) was placed in a previously weighed moisture can and dried for three hours on an oven set at 105℃, 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 Kjedahl method [13]. 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 mls 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 [11,12]. Five gram (5 g) of each sample was wrapped with a weighed porous paper (Whatman filter paper No 40). The wrapped sample was placed in a Soxhlet column flask mounted unto a weighed oil extraction flask containing about 300 ml of petroleum ether (40-60℃ 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 [11,12]. The carbohydrate content was calculated and expressed as the nitrogen free extract (NFE) as shown below:

% 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 [12]. 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℃, 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, Escherichia coli* and *Pseudomonas aeruginosa,* were clinical isolates provided by the Central Laboratory of National Root Crop Research Institute Umudike Abia state, Nigeria.

2.8 Antibacterial Activity Test of the Aqueous and Ethanol Extracts of the Samples

The disc agar diffusion method was used to determine the antibacterial activity of the extracts as reported earlier [14]. Incubation was at 37℃ for 24 hours under aerobic condition. The antibacterial activity was determined by 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 (61.29 %) was lower ($p<0.05$) than that of the seeds (74.34%).

As shown in Table 2, the ash (%) (14.07±0.06) and carbohydrate (%) (53.22±0.05) in the peels was respectively higher ($p<0.05$) than that in the seeds (10.51±0.02, 22.37±0.03) while the fibre (%) (24.09±0.07), fat (%) (0.99±0.08), protein (%) (4.21±0.02) and moisture (%) (3.42±0.03) in the peels was lower ($p<0.05$) than the corresponding content in the seeds (26.31±0.02, 29.62±0.02, 7.41±0.01 and 3.78±0.04).

The aqueous extract of the pawpaw peels and seeds, respectively showed activity (measured as inhibition zone diameter, IZD in mm) against *Escherichia coli* (12.33±1.15, 15.67±1.15), *Staphylococcus aureus* (8.00±1.73, 11.67±1.15) and *Pseudomonas aeruginosa* (7.67±1.15, 0.00±0.00). Beside *P. aeruginosa* that was not susceptible to the aqueous extract of the paw seeds, the activity elicited by the peels extract against *E. coli* and *S. aureus* was significantly ($P<0.05$) lower than that of the seeds (Table 3).

Table 1. Percentage yield of the *Carica papaya* (pawpaw) seeds and peels samples

Samples	Initial or wet weight (g)	Final or dry weight (g)	Yield (%)
Seeds	$175.10±1.12^{a}$	$130.16±1.24^{c}$	$74.34±1.32^{e}$
Peels	$85.54±1.03^{b}$	$52.43±1.41^{d}$	$61.29±1.15^{f}$

Result = Mean ± SD of triplicate determinations. Different superscript in a row or column means that the difference is significant (p<0.05)

Table 2. The proximate content (%) of the *Carica papaya* (pawpaw) peels and seeds

Parameters	Peels (%)	Seeds (%)	Difference (%)
Ash	$14.07±0.06^{e}$	$10.51±0.02^{f}$	±3.56
Fibre	$24.09±0.07^{g}$	$26.31±0.02^{h}$	±2.22
Fat	$0.99±0.08^{c}$	$29.62±0.02^{d}$	±28.63
Protein	$4.21±0.02^{a}$	$7.41±0.01^{b}$	±3.20
Moisture	$3.42±0.03^{i}$	$3.78±0.04^{j}$	±0.36
Carbohydrate	$53.22±0.05^{k}$	$22.37±0.03^{l}$	±30.85

Result = Mean ± SD of triplicate determinations. Different superscript in a row or column means that the difference is significant (p<0.05)

Table 3. Anti-bacterial activity (inhibition zone diameter, IZD (mm) at a concentration of 100 mg/ml of aqueous extract of *Carica papaya* (pawpaw) peels and seeds

Bacteria species	Water extract (100 mg/ml)		Difference (mm) between the peels and seeds extracts
	Pawpaw peels	Pawpaw seeds	
E. coli (IZD, mm) (gram –ve)	12.33±1.15[a]	15.67±1.15[b]	±3.34
S. aureus (IZD, mm) (gram +ve)	8.00±1.73[b]	11.67±1.15[c]	±3.67
P. aeruginosa (IZD, mm) (gram –ve)	7.67±1.15[b]	0.00±0.00[a]	±7.67

Result = Mean ± SD of triplicate determinations. Different superscript in a row or column means that the difference is significant (p<0.05)

As shown in Table 4, the activity (mm) of ethanol extract of the pawpaw peels against *Escherichia coli* (17.33±1.15), *Staphylococcus aureus* (15.00±1.73) and *Pseudomonas aeruginosa* (14.33±1.15) was in each case higher (p<0.05) than that of the pawpaw seeds (14.33±0.58, 0.00±0.00, 9.67±1.15). The ethanol extract of the seeds had no activity against *P. aeruginosa*.

Increasing human health challenges and food shortages necessitated increasing search for plants (and even food and fruit wastes) based diets and drugs. Thus, this study determined and compared the proximate and antibacterial properties of milled *Carica papaya L* peels and seeds to obtain a scientific basis for their use as foods and drugs. The lower moisture in, and percentage yield of, the peels in contrast to the seeds could be an indicator of higher moisture (and possibly other proximate content) loss from the peels than from the seeds [14]. The observation could further suggest higher shelf life of the peels than the seeds [15]. The moisture in the peels and seeds (Table 2) compared with the range (3.81-9.69%) reported earlier [15,16,14] and much lower than that reported by Osabor et al. [17] in the leaves (40.3%) and roots (29.2%) of miracle fruit (*Synsepalum dulcificum*) and by Edem and Dosunmu [18] in *Chrysophyllum africanum* (66.67±0.02%). The comparatively lower moisture content of the samples may have resulted from favourable drying conditions suggested by Egbuonu and Osuji [14] that could be understudied and improved upon.

The higher (p<0.05) ash and carbohydrate contents in the peels than in the seeds, suggests that pawpaw peels may be better source of minerals [19,14] and carbohydrate-related energy. The protein content in either the pawpaw peels or seeds compared with the range (5.6-6.62%) reported by Osabor et al. [17]. The ash content in the seeds and peels was higher than the range (6.70-8.00%) reported by Osabor et al. [17]. The carbohydrate content (%) in either the peels or seeds was lower than that (82.27%) in corn [15] while the carbohydrate in peels compared with that reported by Osabor *et al.* [17]. The lower (p<0.05) fibre, fat and protein in the peels than in the seeds suggests lesser related nutrient value of the peels than of the seeds. In particular, the fibre content in the peels and seeds was higher than that in sweet orange seeds [14], in asparagus bean (5.72±0.14%) [20,21], in African breadfruit (1.55%) and soybean (6.46%) [15] and in watermelon rind and seeds [22] hence could be a better dietary fibre source. The fat content in the peels was

Table 4. Anti-bacterial activity (inhibition zone diameter, IZD (mm) at a concentration of 100 mg/ml of ethanol extract of *Carica papaya* (pawpaw) peels and seeds

Bacteria species	Ethanol extract (100 mg/ml)		Difference (mm) between the peels and seeds extracts
	Pawpaw peels	Pawpaw seeds	
E. coli (IZD, mm) (gram –ve)	17.33±1.15[d]	14.33±0.58[b]	±3.00
S. aureus (IZD, mm) (gram +ve)	15.00±1.73[d]	0.00±0.00[a]	±15.00
P. aeruginosa (gram –ve)	14.33±1.15[d]	9.67±1.15[c]	±4.66

Result = Mean ± SD of triplicate determinations. Different superscript in a row or column means that the difference is significant (p<0.05)

lower while that in the seeds was higher than 2.5±0.2% in asparagus bean [20,21], indicating that the pawpaw seeds could be a rich fat source.

Beside *P. aeruginosa* that was not susceptible to the aqueous extract of the seeds, the activity of the aqueous extract of the peels against *E. coli* and *S. aureus* was lower ($p<0.05$) than that of the seeds which is a pointer that the aqueous extract of the seed may not be a broad spectrum antibiotics. The activity of the ethanol extract of the peels against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* was in each case higher ($p<0.05$) than that of the seeds (Table 4) and compared well with that for various solvent extracts of varieties of mango (*Mangifera indica*) kernel and fairly with the standard antibiotics, tetracycline [23]. Earlier studies have reported antimicrobial, including antibacterial and antifungal, activity of *Carica papaya* seeds [24], peels [3], leaves [25] and other parts [26,27]. However, the aqueous and ethanol extracts of the seeds respectively had no activity against *P. aeruginosa* and *S. aureus,* implying that the seeds extracts from these solvents are not bacteriostatic against these pathogens hence could not be useful as broad spectrum antibacterial [28-30]. This was surprising since papaya seed had bacteriostatic activity against many pathogens including *P. aeruginosa* and *S. aureus* [31]. Result of the proximate contents of the aqueous and ethanol extracts of the samples (which were not determined in this study) could have provided a clue to explaining the higher sensitivity of the tested bacteria to the ethanol extract of the pawpaw peels. Further studies in this direction are therefore warranted. Nevertheless, the higher activity of the ethanol extract of the peels could be explained by the apparently differing phytochemical mix in the seeds and peels extracts [32] due probably to differing extraction properties of the different solvents [33]. This explanation appears consistent with the result of the peels but not with that of the seeds, seemingly suggesting that the seeds may have inherent bioactive constituent and property that limit the solubility of its active components in organic solvent herein used. In particular, the lack of activity of the aqueous extract of the seeds against *P. aeruginosa* was similar to the result of Orhue and Momoh [33] for *papaya* seeds and even leaves, implying that aqueous seeds extract of *papaya* could not be effective against *P. aeruginosa* related ailments. Antioxidant activity and potency [34] and antifungal activity [35] have been reported for *Citrus sinensis* (sweet orange) peels and seeds, warranting similar studies for *Carica papaya* (pawpaw) peels and seeds. This study did not compare the antibacterial result with that of standard antibiotic. This was a noted design oversight and limitation that must be addressed in future similar studies.

4. CONCLUSION

This work has been able to highlight some nutrient and antibacterial properties of *Carica papaya* fruit wastes (peels and seeds). The pawpaw peels could be a durable source for mineral and carbohydrate with broader activity and higher potency against the tested bacteria while the seeds could be a source for protein, dietary fibre and fat, but with limited durability and activity against the tested bacteria. Further studies are therefore required to harness the results of the present study thereby enhancing the use of these hitherto food wastes in diets and drugs to ultimately reduce their environmental burden.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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