Physicochemical and Antimicrobial Evaluations of Food Grade Ash Aqueous extract from Furnace and Charred Plantain Peel and Palm Bunches: A Comparative Approach

This work focused on physicochemical and antimicrobial evaluations of food-grade ash extract from furnace ashed and charred plantain peel and palm bunches. Alkaline solutions obtained from these biogenic wastes and limestone were analyzed for their physicochemical and antimicrobial properties. Results showed that the pH of the solutions was alkaline in nature ranging from 10.04 to 11.51. Limestone extract was highly turbid 0.201 NTU, while limestone contained a lot of impurities with 35.64mg/L total dissolved solids. Potassium Hydroxide (KOH) of furnace ashed plantain peel was significantly (P<0.05) higher than the other samples. The aqueous extracts of these wastes and sodium sesquicarbonate (potash) were screened for the presence of their antimicrobial activities against the bacteria and fungi isolates. Microbial isolates used for the study was Bacillus substilis, Pseudomonas aerugenosa, Proteus sp, Staphylococcus aureus, Aspergillus fumigatus Candida albicans, Candida pseudotropicalis and Penicillium expansuum. Charred plantain peel, palm bunch extracts, and limestone inhibited the growth of these microbial isolates. Furnace ashed samples of both plantain peel and palm bunch could not inhibit the microorganisms. Commercial potash extract, which was purchased in an open market, could not also inhibit the organisms. Commercial antibiotics Gentamycin (antibacterial) and Ketoconazole (antifungal) were used as positive controls in this study.

Keywords: Ash, Potash, Bio-carbonate, Limestone, Aqueous, fermented

INTRODUCTION

Ash is the mineral residue obtained after the combustion of organic materials. Ash is obtained after complete removal of all moisture and organic materials such as fats, protein, carbohydrates, vitamins, and organic acids, etc. by incineration (either by open pan combustion or muffle furnace combustion) of the combustible plant materials. Plant materials are gotten from cultivable and wild vegetables which are important sources of minerals and vitamins (Adewuyi et al., 2008) most of which are undervalued and regarded as waste. Such plant materials include palm fruit bunches, plantain peels, banana leaves, palm inflorescence, wood plant, bark, wood sawdust, leaves, woody debris, pulp, husk, groundnut peels, palm fronds, cocoa pods and others which contains a good percentage of potash and soda ash. (Babayemi et al., 2010; Uzodinma et al., 2014). They also contain oxides of potassium, sodium, calcium that yield their corresponding hydroxides upon dissolution in water (Babayemi et al., 2010; Uzodinma et al., 2014). To exploit these, quantification and utilization becomes the best alternative. Minerals are also present in all body tissues and fluids which presence is necessary for the maintenance of certain physicochemical processes essential for life.

In Nigeria, food ashes have traditionally been incorporated into the human diet. For instance, in Amuda, Isuochi in Umunneochi Local Government Area of Abia State, Nigeria, the filtrate obtained from a mixture of plant ash and water (brown colour and slippery indicating alkalinity)
can be used in making 'Ngu', an emulsion of oil in water, (Udeotok, 2012). Plant ash can also be used for preparing traditional foods such as ighu, ugba, isiewu, nkwo, abacha (Irvine, 1961) and as a tenderizer in cooking foods like African breadfruit, meat, corn and hard to cook legumes. In Annang tribe of Akwa Ibom State, Nigeria, filtrate from palm bunch ash and water is used in preparing a local delicacy known as “Otong" used in eating meat, drinking palm wine and as a spice for a soup to prevent the soup from having a bitter taste (Udeotok, 2012).

Antimicrobials are compounds that can inhibit the growth of pathogenic microorganisms (bacteriostatic effects) or kill bacteria (bactericidal effects) that come in contact with the antibiotic. Synthetic antimicrobial compounds have a negative effect on animals, including cattle and the humans who consume them, such as leading to increase drug dosages and other side effects. Animals given antibiotics cannot be slaughtered within a certain time of treatment and antibiotics increase microbial resistance to certain drugs. The increase in cases of pathogenic bacterial resistance has hastened the search for new sources of antimicrobial compounds through the development of ways to utilize natural medicinal substances that have antimicrobial activity. Antibiotics obtained naturally from microorganisms are called natural antibiotics. Some types of plants that have natural antimicrobial activity include onions, garlic (Allium sativum L.) and leeks (Allium porrum L.), ginger, honey and Capsicum annuum L. The use of natural antimicrobials is expected to minimize negative effects on livestock animals and the humans who consume them. Oil palm bunches are oil palm plantation waste material that can be utilized as feed and have antioxidant activity. The potential antimicrobial activities of plantain peel and palm bunch extracts have not been widely reported. The objective of this study was to extract and identify antimicrobial compounds in aqueous extracts of oil palm bunch and plantain peel that are potentially useful as natural antibiotics and that may be used as a substitute for synthetic antibiotics.

**MATERIALS AND METHODS**

**Collection of Raw Materials**

Unripe plantain peels were collected from Food Affairs Limited, 98 ikot Etukene Road and Oil palm bunches from oil palm mill at ikot Ayan Itam, Itu Local Government Area. Crude palm oil was obtained from Agro-Ideas, Ibesiikpo Local Government Area and Limestone (Sodium sesquicarbonate) were purchased at Etaha Itam Market, Itu Local Government Area all in Akwa Ibom State and transported to the Department of Food and Science and Technology Processing Laboratory for onward processing and analysis.

**Extraction of Ash from Oil Palm Empty Fruit Bunch**

The method of Ogunsuyi and Akinawo (2012) was used for the extraction process with some modifications. Two thousand grams (2000g) of the collected palm bunch wastes were sundried and later oven-dried at 105°C for two days to ensure adequate removal of moisture from the sample. The bunch was said to be “bone-dried”. One thousand grams (1000g) of the bone dry bunches were charred for 1 hour to ensure uniform combustion which yielded about 100g of charred ash.

Another, 1000g of the dried palm bunch was ground with mortar and pestle to increase its surface area and was burnt in a temperature-controlled furnace (Model 5XL) set at a temperature of 550°C for proper ashing which lasted for about 6 hours which yielded approximately 70g of ash. The ashed samples were homogenized by crushing between fingers and then sieved with an analytical sieve of mesh size of 0.105mm (US sieve No. 140 Tyler equivalent 150 Mesh) to obtain particle of uniform size.

**Extraction of Ash from Unripe Plantain Peel**

Two thousand (2000) gram of the plantain peels waste was washed with distilled water and sundried for three days to constant weight. The peels were sun-dried and later oven dried at 105°C for two days to ensure adequate removal of moisture from the sample. The drying continued until it became "bone-dried". One thousand grams (1000g) of the bone-dried peels were charred for 1 hour to ensure uniform combustion, which yielded about 100g of charred ash (Umeh and Maduakor, 2013). The ash samples were powdery but it was sieved with 0.105 mm sieve (US Sieve No. 140 Tyler equivalent 150 Mesh) to remove large particles.

Another, 1000g of the dried peels was ground with mortar and pestle. The grounded waste peels were burnt in a temperature-controlled furnace (Model 5XL) set at temperature of 550°C for about 6 hours for proper ashing yielding approximately 70g of ash. This was cooled in desiccators before being sieved with a mesh size of 0.105 mm (US sieve No. 140 Tyler equivalent 150 Mesh).

**Preparation of the Aqueous Crude Extract from Limestone**

The limestone was ground into a fine powder using laboratory mortar and pestle, after which and 2.5 grams of the limestone powder was made up to 100ml with distilled water to make a 2.5% solution. The flask was covered with aluminum foil paper and left for 3 weeks (21 days) for digestion. After this period, the flask was then placed on an electric mechanical shaker and shaken for 4 hours. After this, the flask was allowed to stand for 48 hours and the content was filtered using poplin cloth and re-filtered with Whatman No.1 filter paper (125cm) to obtain a clearer extract.
Preparation of the Aqueous Extract from the Resulting Ashes

Twenty-five (25) grams of the ash was placed in a 1 liter round bottom flask and made up to 1000ml with distilled water. The flask was covered with aluminum foil and left for 3 weeks (21 days) for digestion. After this period, the flask was then placed on an electric mechanical shaker for 4 hours. After this, the flask was allowed to stand for 48 hours and the content was filtered using poplin cloth and re-filtered with Whatman No.1 filter paper of (125cm) to obtain clearer extract.

Physicochemical Analysis of the Extracts

Determination of Turbidity

Turbidity was carried out by using HANNA Instruments Turbidimeter Model P17WT following the method of AOAC (2010). The turbidity was measured as Nephelometric Turbidity Units (NTU).

Determination of pH

Digital pH Meter produced by HANNA Instruments Model STD218F was used in this study. The glass electrode was thoroughly wetted with distilled water. The pH meter was switched on and was standardized. This was done by connecting a glass electrode to the pH meter and inserting the electrode into the buffer solution. This was allowed to stabilize and pH meter reading indicated 4.0, which is equal to its known value. The same thing was repeated using a buffer solution with a pH of 9.0. The pH of the samples was tested by inserting the electrode into it. This was then allowed to stabilize and the readings were recorded.

Total Dissolved Solid (TDS)

Fifty (50) ml of previously filtered potash after digestion was stirred and transferred into a pre-weighed evaporating dish. The filtrate was heated for an hour in an oven. The evaporating dish was cooled in a desiccator to balance the temperature and weighed. This procedure was repeated until a constant weight was obtained (APHA, 2005).

Colour Measurement

The colour of the prepared emulsion otherwise known as Otong was measured directly with eyesight and the colour physically seen was recorded for each sample accordingly.

Anti-Microbial Analysis

Test Organisms

Microorganisms used in this study were isolated from saponified dishes (otong) produced with ash solutions of palm bunch, plantain peel, limestone using commercial potash as control, four bacterial isolates were isolated from these saponified dishes which was used in the preparation of isiewu (goat heat delicacy with fermented pentaclethra macrophylla) including; Bacillus subtilis, Pseudomonas aeruginosa, Proteus sp, Staphylococcus aureus and four fungal isolates including Aspergillus fumigatus Candida albicans, Candida pseudotropicalis and Penicillus expansuim. The strains were from the stocks of pure culture collections maintained in the Mckanty stock bottle stored in the refrigerator.

Disc Diffusion Method

The different extracts from plantain peel, oil palm empty bunch and limestone were assayed for their antimicrobial activity using the disc diffusion method. The discs were prepared as described by Isu and Onyeagba, (1998). Sterile paper discs (Whatman No. 1 filter paper) of seven-millimeter (7mm) diameter were impregnated with the different extract concentration for 2 hours. The culture medium (Muller Hinton Agar) used was carefully prepared according to the manufacturer’s instruction. This was a commercial product of Oxoid Ltd Company, England. The Agar plates were seeded with 0.1 ml broth culture of test organisms and the impregnated discs were placed on the center of the plates and allow for diffusion and incubated at 37°C for 18-24 hours. Gentamycin was used as a positive control for bacteria and Ketoconazole as a positive control for fungi. They were observed for their clear zone of inhibition and measured with a transparent meter ruler.

Statistical Analysis

Statistical Package for Social Science (SPSS, version 20) was used for the statistical analysis. The differences between samples in each parameter tested were done using One Way Analysis of Variance (ANOVA) and New Duncan's Multiple Range Test as a post-hoc test when the analysis of variance indicates a significant difference in their means. A significant level of P<0.05 was used throughout the study.

RESULTS AND DISCUSSION

Physicochemical Analysis of the Ash Solution

The results of the physicochemical properties of the ash solutions from different wastes are presented in (Table 1). The pH of ash solutions was in the range of 10.04-11.51, which suggests good alkalinity in the production of a good emulsion base, required for otong delicacy. This agrees with the reports of Uzodinma et al., (2014); Udoetok, (2012); Israel and Akpan, (2016), that ashes are usually alkaline (pH >10) because they are composed primarily of calcium carbonate, potassium chloride, and sodium chloride. Andrews (1983) reported that plant ash is occasionally utilized as a substitute condiment when common salts or sea salts are unavailable.
The turbidity of the furnace ashed samples was significantly lower than charred samples (See Table 1). The low values obtained in furnace ash solutions may be due to the complete combustion of the blackish particles (suspended solids) and other components such as lignocelluloses, phenolic, oil, etc. Turbidity is associated with impurity, which is solely dependent on the degree of combustion.

Result obtained from (Table 1) revealed that, there was a significant difference (P<0.05) between total soluble solids of all the samples. Charred plantain peel was found to be higher in total soluble solid followed by charred palm bunch and furnace plantain peel with furnace palm bunch having the least total soluble solid. Furnace ashed plantain peel and palm bunch ash solutions recorded the lowest values after 21 days of extraction. This suggests that the extraction of soluble constituents from the ashes increases with soaking (digestion) time. High values of total soluble solids in charred samples may be attributed to the impurity levels of their ashes. Potassium hydroxide concentration (KOH) of the solution was found to be higher in furnace ashed samples when compared with potassium hydroxide concentration (KOH) by charred samples. This is because the major components of plantain and palm bunch residues (Lignocelluloses, Phenolic and oils, etc) oxidizes into the gaseous emission during incineration leaving behind metal oxides and other elemental components that form a good alkaline solution. The low values of potassium hydroxide (KOH) in charred samples can be attributed to incomplete combustion of the blackish particles.

The brown colours obtained in charred samples are indication of suspended solids (cloudiness) resulting from incomplete combustion during charring compared with the white colouration obtained in furnace ashed samples, which could have been due to the complete combustion of the blackish particles. Also, the dark brown colouration obtained after digestion of limestone may be due to impurities of the mineral salt and also handling. The results of this study are in agreement with the reports of Babayemi et al., 2010; Uyigue et al., (2013), and Udoetok, (2012).

### Table 1: Physicochemical composition of the Food-grade Ash Solutions from the different ashes

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Turbidity (NTU)</th>
<th>Total Dissolved Solid (mg/L)</th>
<th>KOH (g/dm³)</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPEL₁</td>
<td>11.51 ± 0.01</td>
<td>0.089 ± 0.001</td>
<td>16.78 ± 0.02</td>
<td>29.40 ± 0.05</td>
<td>White</td>
</tr>
<tr>
<td>PPEL₂</td>
<td>11.38 ± 0.02</td>
<td>0.118 ± 0.002</td>
<td>22.42 ± 0.04</td>
<td>15.26 ± 0.04</td>
<td>Light brown</td>
</tr>
<tr>
<td>OPE₁</td>
<td>11.36 ± 0.03</td>
<td>0.100 ± 0.000</td>
<td>14.51 ± 0.01</td>
<td>27.20 ± 0.03</td>
<td>White</td>
</tr>
<tr>
<td>OPE₂</td>
<td>10.70 ± 0.05</td>
<td>0.116 ± 0.002</td>
<td>20.66 ± 0.03</td>
<td>14.91 ± 0.01</td>
<td>Light brown</td>
</tr>
<tr>
<td>LMSE</td>
<td>11.04 ± 0.01</td>
<td>0.201 ± 0.001</td>
<td>27.30 ± 0.05</td>
<td>25.24 ± 0.02</td>
<td>Deep brown</td>
</tr>
<tr>
<td>COMM</td>
<td>10.04 ± 0.04</td>
<td>0.154 ± 0.001</td>
<td>35.64 ± 0.04</td>
<td>20.14 ± 0.01</td>
<td>Deep brown</td>
</tr>
</tbody>
</table>

Values are mean ± Standard deviation of triplicate determinations, means with the same superscript on the same column are significant (p<0.05) different.

PPEL₁ -(Aqueous extract of furnace ashed plantain peel)
PPEL₂ -(Aqueous extract of charred plantain peel)
OPE₁ -(Aqueous extract of furnace ashed palm bunch)
OPE₂ -(Aqueous extract of charred palm bunch)
LMSE- (Aqueous extract of limestone)
COMM- Commercial potash extract purchase from Ikot Ekpene local market,
CONTROL-Gentamycin (an antibacterial agent)

**Antimicrobial activity of the potash from the different substrates on the microbial isolates:**

Assessment of the antimicrobial activities of the various extracts (plantain peel, palm bunch, commercial potash and limestone) on the selected microbial isolates (Bacillus substilis, Proteus sp, staphylococcus aureus, Pseudomonas aeruginosa, Asperigillus fumigatus Candida albicans, Penicillium expansuium Candida pseudotropicalis) (See Table 2 & 3), revealed that the charred plantain peel ash compete effectively with the commercial antimicrobial agents (Gentamycin and Ketoconazole for bacteria and fungi respectively) used in inhibiting the growth of the test isolates. Antimicrobial activity was not observed from all the extracts. Charred plantain peel extract has a surface-active ingredient known as surfactant, which was suspected to be responsible for the inhibitory effect observed against some test organisms.

Furnace ashed extract of plantain peel (PPEL₂) was able to inhibit only Bacillus substilis but was not able to inhibit other organisms. Furnace ashed samples of plantain peel and palm bunch were found not to be effective against this test isolates except for furnace ashed plantain peel, which was able to inhibit only one microbial isolate (Bacillus substilis). This could be as a result of the complete combustion of the phytochemicals and other surface-active ingredients or materials contained in this plant substrate during furnace ashing at a temperature of 550°C. After ashing in the furnace, a white solution was obtained upon digestion and leaching leaving only the oxides. The inhibitory activity observed in charred samples could be attributed to incomplete combustion of the plant materials or phytochemicals, which are responsible for the antimicrobial effect. The antimicrobial activity of plantain peels (Musa paradisiaca) has been reported by previous investigators. This work is in line with Ahmad and Beg.
Limestone (sodium sesquicarbonate) extract, which was obtained after twenty-one (21) days of wet digestion, showed an inhibitory effect by competing with the charred plantain peel extract (See Table 2 & 3). Kemka-Evans et al., (2013) in his study, reported that Potassium carbonate (limestone) showed the highest antimicrobial effect on the test bacteria (streptococcus spp and Bacillus spp). It was also observed in their work that Bacillus spp. was more resistant to the antimicrobial agents showing susceptibility only to the soxhlet and aqueous extracts of Potassium carbonate. This report agrees favorably with the findings of this work. Extract of palm bunch ash had no significant (P>0.05) effect on the test isolates, this may have been because palm bunch extract has not been reported to possess inhibitory properties necessary to inhibit the organisms. Kemka-Evans et al., (2013) who studied the palm husk reported that extract from palm husk was able to inhibit streptococcus sp. and Bacillus sp. which contradict with the result of palm bunch ash extract in this study because palm husk is still from the palm tree which palm bunches are gotten.

Similarly, the commercially acquired potash from a local market in Ikot Ekpene had no positive effect on the test organisms hence any inhibition observed, while the commercial potash could not inhibit the organisms may be attributed to the production procedures adopted and the plant materials used by the market women. Since the marketers of this commercial potash did not disclose the constituents of their formula, it is suspected that the commercial potash was prepared using palm bunches burnt where other constituents (like charcoal, insect, etc.) would have contaminated it, which made the solution highly impure.

The two commercial antibiotics Gentamycin and Ketoconazole for bacteria and fungi respectively used as controls were able to inhibit these organisms when compared with alkaline solutions obtained from the furnace and charred plantain peel, palm bunch, limestone and commercial potash. The antimicrobial activity of the various extract obtained from furnace ashed and charred plantain peel, palm bunch, limestone and commercially acquired potash from the market showed varying magnitudes of inhibition patterns with standard drug gentamycin and ketoconazole which are a well-known broad-spectrum antimicrobial agent. The differences in the antimicrobial activities of the various extracts may be due to varying the active constituents in the plant substrate used. The difference in potency may be due to the stage of collection of the plant sample, different sensitivity of the test strains and method of extraction after digestion (Nimri et al., 1999). Plant extracts are becoming popular as potential sources of antimicrobials and several reviews have been written (Rojas et al., 1992). Since the charred plantain peel ash extract appears to have a broad antimicrobial activity spectrum, it could be useful in antiseptic and disinfectant formulations as well as in chemotherapy.

Food spoilage is a common problem faced all over the world. High temperatures, humidity, unhygienic preparation of food, lack of proper storage, etc. all contribute to food spoilage and growth of pathogenic bacteria that cause food illness. The chemical antibiotics have side effects on the host and also destroy the natural microflora of the body. In contrast, the natural plant extracts offer a very safe medical treatment with no side effects.

The antimicrobial activity trend of the extracts observed in this study was as follows; Control>Charred plantain peel ash> Limestone> Charred palm bunch> Furnace ashed plantain peels >Furnace ashed palm bunch >Commercial potash.

---

Table 2: Antibacterial activities of different potash on some organisms Zone of inhibition (mm)

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>PPEL</th>
<th>PPEL&lt;sub&gt;2&lt;/sub&gt;</th>
<th>OPEB</th>
<th>OPEB&lt;sub&gt;2&lt;/sub&gt;</th>
<th>LMSE</th>
<th>COMM</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>11.2</td>
<td>7.9</td>
<td>9.2</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>14.2</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>9.2</td>
<td>-</td>
<td>10.1</td>
<td>-</td>
<td>9.8</td>
<td>-</td>
<td>13.6</td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>10.4</td>
<td>-</td>
<td>9.4</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>13.8</td>
</tr>
<tr>
<td>Pseudomonas aerugiosa</td>
<td>10.2</td>
<td>-</td>
<td>8</td>
<td>-</td>
<td>8</td>
<td>-</td>
<td>15.4</td>
</tr>
</tbody>
</table>

- = No inhibition
>7mm = Inhibition
PPEL<sub>1</sub> -(Aqueous extract of furnace ashed plantain peel)
PPEL<sub>2</sub> -(Aqueous extract of charred plantain peel)
OPEB<sub>1</sub> -(Aqueous extract of furnace ashed palm bunch)
OPEB<sub>2</sub> -(Aqueous extract of charred palm bunch)
LMSE- (Aqueous extract of limestone)
COMM- Commercial potash extract purchase from Ikot Ekpene local market,
CONTROL- Gentamycin (an antibacterial agent)
CONCLUSION

The outcome of this work suggests that, *Musa paradisiaca* peel and *Elaeis guineensis* bunch ash extracts can be used favourably in the preparation of “otong” delicacy (goat head) other than the more conventional method of using potassium carbonate (limestone potash) in the South-South and South-East regions of Nigeria. This will discourage the use of limestone (*akanwu*) or baking soda in making emulsion for local delicacies. This study may be useful in the production of food fortificants for local delicacies in the food industries and food supplements.

The microbes against which the extracts were effective are pathogens already implicated in the etiology and severity of human diseases. Thus the plant extracts of plantain peel and palm bunch both furnace and charred ash may be useful in pharmaceutical and medical formulations. However, the possibility of further purification and formulation into antibiotics may be considered later.

CONFLICT OF INTEREST

The authors declares no conflict of interest

REFERENCE


Accepted 29 January 2020


Copyright: © 2020. Ntukidem et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are cited.