GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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# Evaluation of the phytochemical, mineral components and antimicrobial activity of empty palm fruit bunch ash and unripe plantain peel ash for use as alkaline bio activators of charcoal

Ezinne Oluoma Ozoani *, Uzoamaka Ogechi George-Okafor and Uloma Ezinwanne Ozoani

*Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology, Enugu, Nigeria.*

GSC Biological and Pharmaceutical Sciences, 2024, 28(02), 146–152

Publication history: Received on 30 June 2024; revised on 08 August 2024; accepted on 10 August 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.28.2.0258

## Abstract

Empty palm fruit bunch ash (EFBA) and Unripe plantain peel ash (PPA) are obtained by converting dried empty palm fruit bunch and dried unripe plantain peel into ashes by either controlled open burning or the use of furnace. Chemical activators are mostly used to activate charcoal. However, these chemicals are not affordable and unsafe for use in food industries as they can pose serious health issues in humans. This led to the aim of this study which was to evaluate the phytochemicals, mineral constituents and antimicrobial properties of EFBA and PPA as potential alkaline bio-activators for Neem charcoal. The qualitative phytochemical analysis of both EFBA and PPA was done using conventional methods, mineral constituents were determined using Atomic Absorption Spectrophotometer (AAS). The ashes were exposed to antimicrobial susceptibility test against test bacterial isolates (*Staphylococcus aureus* strain OPD001-1; *Salmonella enterica* subsp*. enterica serovar derby* strain RM005) and fungal isolates (*Aspergillus niger* strain ND 89; *Aspergillus flavus* strain Beca-67). The result of the phytochemical screening revealed the presence of Alkaloids, Carbohydrates, Cardiac glycosides and Saponins in both ashes while Fructose, Resins and Terpenoids were present in PPA. The mineral constituents were in high quantities in both ashes with Potassium highest in EFBA (760531.80mg/kg) and the least being Calcium in EFBA (821.00mg/kg). EFBA and PPA were both resistant to the test bacterial and fungal isolates. However, the mineral composition of both ashes suggests their potentials as alkaline bio-activators for Neem charcoal.

**Keywords:** EFBA; PPA; Charcoal; Bio-activator; Antimicrobial.

## 1. Introduction

Empty palm fruit bunch (EFB) is the outer layer where palm fruits are removed from, they are usually fibrous and regarded as the most significant solid waste generated during palm oil production by palm oil processing industries (Hisham and Jamil, 2020; Hardinato *et al*.,2023). In Nigeria, EFB are often discarded as wastes because of its high moisture and bulky nature that makes it difficult to be handled and transported. These wastes constitute environmental pollution as a result of its decomposition which produces gases (Obada *et al*.,2023; Igwe and Onyegbado,2007). Several researchers have explored different methods by which EFB can be converted into useful products and possibly add value to it. One of the methods is its conversion to ash which would reduce its volume and make its application easier (Okoli, 2010).

Empty palm fruit bunch ash (EFBA) is obtained by conversion of EFB into ashes either by open burning or the use of furnace (Okoli,2010). EFBA has been reported to contain some minerals like; Calcium(Ca), Potassium(K), Sodium(Na), Magnesium(Mg), Phosphorus (P) (Duruanyim *et al*., 2016; Udoetok, 2012; Nwoka *et al*.,2021; Jamadhi *et al*., 2020). In south eastern part of Nigeria, the filtrate from EFBA is called "Ngu" and used as a food additive in preparing traditional delicacies and also as meat tenderizer (Nwoka *et al*.,2021), similarly the Annang tribe of Akwa Ibom State, Nigeria use

* Corresponding author: Ezinne Oluoma Ozoani

it in preparing their local delicacy known as "Otong" (udoetok,2021). Other applications of EFBA includes; as organic fertilizer for the improvement of soil fertility and nutrients (Ojeniyi *et al*.,2009; Okoli, 2010), treatment of land to improve plant yield (Thaiwe, 2008), treatment of water and wastewater (Chiew and Shimada, 2013), as adsorbents for the removal of heavy metals in aqueous solutions (Lee *et al*., 2017; May *et al*., 2019), as natural =coagulant in the removal of COD and TSS in palm oil mill effluent (Nur-Syahira and Zadariana, 2020). It has also been used to produce solid soap (Udoetok,2012).

Unripe plantain peels are gotten after the plantain fruits are removed and it constitutes about 40% of the fruit (Adetayo and Olatunji, 2019). The plantain peels like the EFB are often disposed in farmlands and dumpsites which also contributes to environmental pollution. The plantain peel ash (PPA) is produced by burning the dried peels in an open fire or the use of furnace at a temperature range of about $400^0$C -$800^0$C (Olabanji *et al*.,2012; Igboegwu, 2013). PPA has been reported to contain some mineral elements such as; Calcium (Ca), Potassium(K), Sodium (Na), Magnesium (Mg),Phosphorus (P) (Olabanji *et al*., 2012; Adbulahi *et al*.,2022). There are several reports on the use of PPA for different purposes. Omoniyi *et al*., (2019) explored the potential of plantain peel ash as a potash bio-catalyst for the production of reducing sugar. It has been reported as an effective concrete admixture in construction (Adetayo and Olatunji, 2019; Abdullahi *et al*., 2022). Falowo *et al*., (2022) synthesized base catalyst from ripe and unripe peel ash for the transesterification of waste cooking oil, it has also been used as a source for alkali for soap production (Onyegbado *et al*.,2002). Leached potash from PPA was used as alkaline bio-activator for the activation of coconut shell carbon, the results obtained compared favorably with chemically activated carbon (Efovbokhan *et al*., 2019).

The use of chemicals as activators for charcoal is the conventional method, however, these chemicals are expensive and unsafe for use in food because of the chemical deposits which are detrimental to health of humans. This led to the search for alternative biological agents that are cheaper, safer, and readily available. This study was aimed at screening for phytochemical, mineral constituents and antimicrobial susceptibility of EFBA and PPA as potential alkaline bio-activators for Neem charcoal.

## 2. Materials and Methods

### 2.1. Sample collection

- unripe plantains were purchased from new market, Enugu, Enugu State, Nigeria.
- Empty palm fruit bunch was gotten from palm oil processing plants in Enugu Ngwo, Enugu State, Nigeria.

The test bacterial isolates (*Staphylococcus aureus* strain OP0001-1 ,*Salmonella enterica* subsp. *enterica serovar derby* strain RM 005) and fungal isolates (*Aspergillus niger* strain ND 89, *Aspergillus flavus* strain Beca-67) were gotten from Department of Microbiology and Brewing, Enugu State University of Science & Technology, Enugu State, Nigeria.

### 2.2. Preparation of Empty palm fruit bunch ash (EFBA)

The method of Nelson *et al*., (2023) was used. The empty palm fruit bunch was washed to remove soil and debris and was kept to dry at room temperature for 4weeks; after drying it was slowly burnt for 30mins to obtain the ash, the ash was sieved with o.8mm sieve to get rid of impurities. The sieved ash was carbonized in the absence of oxygen in a Muffle furnace at $800^0$C for 30mins, it was stored in a sterile zip lock bag after cooling at room temperature and labeled EFBA.

### 2.3. Preparation of Unripe plantain peel ash (PPA)

The method of Efeovbokhan *et al*., (2019) was used. The unripe plantain was peeled and the peel was kept to dry at room temperature for 4weeks. The dried peel was burnt to obtain the ash, the ash was sieved using 0.8mm sieve to get rid of impurities. The sieved ash was carbonized in the absence of oxygen in a Muffle furnace at $800^0$C for 30mins, it was stored in a sterile zip lock bag after cooling at room temperature and labeled PPA.

### 2.4. Qualitative Phytochemical Assays of the EFBA and PPA

The ashes were screened for the following phytochemicals using the conventional methods as described by Okanlawon *et al*., (2023)

Alkaloids, Amino acids, Protein, Carbohydrates, Polysaccharide, Fructose, Flavonoid, Cardiac glycosides, Phenol, Reducing sugars, Resins, Saponins, Steroids, Tannin, Terpenoids and Oil.

### 2.5. Determination of some mineral constituents of EFBA and PPA

The calcium (Ca), sodium (Na), magnesium (Mg), potassium (K) and phosphorus (P) contents of the ashes were determined using the method of Duruanyim *et al*., (2016). In each of the ashes, 200mg were weighed into a flask and 20mls of concentrated nitric acid ($HNO_3$) was added in each of the flask thereafter, the samples were predigested by gentle heating for 20mins after which the digestion was continued for 40mins and was stopped when a clear digest was obtained. The content was allowed to cool for about 20mins and was filtered into a 50ml volumetric flask using Whatman no.42 filter paper (150mm) diameter. The filtrate was passed through the nebulizing Atomic Adsorption Spectrophotometry (AAS) where the concentration of the various minerals was determined in mg/kg.

### 2.6. Antibacterial activity of EFBA and PPA

The antibacterial activity of the alkaline bio activators (EFBA and PPA) was done using modified agar well diffusion methods of Iotsor *et al. (*2019). The bacterial isolates used were *Staphylococcus aureus* strain 0P0001-1, *Salmonella enterica subsp. enterica serovar derby* strain RM 005.

Each of the bacterial isolate was prepared by sub culturing the isolate onto fresh Nutrient agar plate and incubated at $37^0$C for 24hours to obtain 24hr culture. A loop full equivalent to 5 colonies of the 24hour culture was diluted in 3ml normal sterile water to obtain a density comparable to 0.5 McFarland standard turbidity corresponding to about $1.5 \times 10^8$ colony forming unit (CFU) per ml. The bacteria suspensions were further adjusted to standard by diluting the density to 0.1 at 600nm using Spectrophotometer (Jenway 6105UV/V,50 H.Z/60HZ). One millilitre of the adjusted bacteria suspension was spread over the surface of Muller Hinton Agar plates using sterile swab sticks and allowed to settle for 10minutes. Thereafter, Sterile cork borer of 6mm was used to bore holes on the cultured Muller Hinton Agar plates. Five grams each EFBA and PPA were weighed into sterile beakers respectively, 5mls of sterile water was added to it to make a 100% concentration to obtain a working solution. Each of the bio-activators, 0.5mL of the working solution of EFBA and PPA were dispensed into the holes and allowed to diffuse into the agar for one hour before they were incubated at $37^0$C for 24hours. The zones of inhibition were measured in mm.

### 2.7. Antifungal activity of EFBA and PPA

Modified poisoned food technique method as described by George-Okafor *et al*. (2020) was adopted. The test fungal isolates used were; *Aspergillus niger* strain ND 89 and *Aspergillus flavus* strain Beca-67.

In each of the prepared working solution (100% concentration) of EFBA and PPA , 2mL was poured into sterile petri dishes followed by addition of 20mL respectively of already prepared Potato Dextrose Agar (PDA) and mixed gently in a circular motion and allowed to solidify. Then, a 5mm diameter mycelia disc was made on a 7-day old culture using sterile 5mm diameter cork borer. Each mycelial disc was aseptically placed with the mycelial facing downward at the centre of each of the petri dishes and incubated at $37^0$C for 7days. The plates without the Bio-activators and Neem charcoal served as control. The growth of the mycelium was measured using Vernier caliper and calculated in percentage as stated below:

$$\%\text{Inhibition} = \frac{C - T}{C} \times 100$$

Where C was the growth of the mycelium in control and T was the growth of mycelium in the samples set.

## 3. Results

**Table 1** Qualitative Phytochemical Profile of the Bio-activators

| **Bio-activators** | **Phytochemical Components** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AL | AA | PT | CD | PS | FT | FL | CG | PH | RS | RN | SP | ST | TN | TP | OL |
| Empty palm fruit bunch ash (EFBA) | + | ND | ND | +2 | ND | ND | ND | +3 | ND | ND | ND | +2 | ND | ND | ND | ND |
| Unripe plantain peel | +2 | +3 | ND | +3 | ND | +3 | ND | +2 | ND | +2 | ND | +2 | ND | ND | +2 | ND |

key:AL= Alkaloids, AA= Amino acids, PT=Protein, CD= Carbohydrates, PS= Polysaccharide, FT= Fructose, FL= Flavonoids, CG= Cardiac glycosides, PH= Phenols, RS= Reducing sugars, RN= Resins, SP= Saponins, ST= Steroids, TN= Tannins, TP= Terpenoids, OL= Oil; ND= Not detected ; + = Scanty content; +++ = High content

**Table 2** Some Mineral Constituents of Alkaline Bio-activators.

| **Mineral Elements(g/kg)** | **Sample type** | |
|---|---|---|
| | EFBA | PPA |
| Calcium | 0.821 | 4141.20 |
| Potassium | 694.13288 | 760.5318 |
| Sodium | 21.26522 | 203.40065 |
| Magnesium | 11.69833 | 7.18148 |
| Phosphorus | 16.90117 | 17.82952 |

Key:EFBA= Empty Palm Fruit Bunch Ash; **PPA= Unripe Plantain Peel Ash**

**Table 3** Preliminary Potential of the Bio-activators against Microbial Isolates

| **Bio-activators** | **Mean zone of Inhibition (mm/%)** | | | |
|---|---|---|---|---|
| | Bacteria | | Fungi | |
| | *Staph. aureus* | *Salmonella enterica* | *A. niger* | *A.flavus* |
| EFBA | - | 6 | 23.7 | - |
| PPA | - | 10 | - | 9.1 |

Key - = Resistant; Staph. aureus= Staphylococcus aureus strain 0PD001-1; Salmonella enterica= Salmonella enterica subsp. Enterica serovar derby strain RM005; A.niger = Aspergillus niger strain ND 89;A.flavus= Aspergillus flavus strain Beca- 67

## 4. Discussion

Phytochemicals are non-nutritive plant chemicals that possess antimicrobial, anti-inflammatory, anti-hypertensive and anti-diabetic properties (Ayoola *et al*.,2008; Oikeh, 2015). The qualitative phytochemical screening result of the bio-activators (EFBA &PPA) as shown in table 1, revealed the presence of Alkaloids, Carbohydrates, Cardiac glycosides and Saponins which were common amongst the bio-activators . However, Amino acids, Fructose, Reducing sugar and Terpenoids were only detected in PPA while, Protein, Polysaccharide, Flavonoid, Phenol, Resins, Steroids, Tannin and Oils were not detected in both ashes. The result of the phytochemical composition of EFBA in this study are similar to the report of Al-Gorany *et al*., (2020) who also detected Carbohydrates and Saponins but differ in Tannin and Phenolic compounds, similarly,Uzairu and Kano (2021) also reported the presence of Saponins and Tannin in PPA which also differed with the present study. The variations in phytochemical composition could be attributed to the variations in

species, methods of extraction, geographical location (Oikeh,2015) and combustion temperature of these ashes (Duruanyim *et al*.,2016).

According to Khan *et al*., (2009) the major mineral elements in ash includes; Calcium (Ca), Potassium(K), Sodium (Na), Magnesium (Mg), Phosphorus (P). Some mineral constituents of EFBA and PPA as shown in table 2 indicated high concentrations of Potassium(K), Calcium (Ca), Sodium (Na), Magnesium (Mg) and Phosphorus (P). There were higher concentrations of K, Ca, Na, and P in PPA while Mg was only higher in EFBA. These results agree with the findings of (Duruanyim *et al*.,2016; Okoli *et al*.,2014; Uzairu and Kano, 2021) who reported a high concentration of these minerals in EFBA and PPA. The variations in the composition of these minerals is largely dependent on the species of the plants, growth conditions, fraction of the ash, soil type and climate (Iwu e*t al*., 2013; Okoli *et al*.,2014; Dermibas, 2005). The high concentration of these minerals especially Potassium suggests it can be an effective alkaline bio-activator for activation of charcoal.

The bio-activators were screened for their antibacterial potentials against the test bacterial isolates (*Staphylococcus aureus* strain OPD001-1 and *Salmonella enterica* subsp.*enterica serovar derby* strain RM 005). The sensitivity of the test isolates to the bio-activators were assayed by measuring the zone of inhibition (ZOI) in (mm). The results of the screening as shown in table3 indicated EFBA was resistant to *Staph. aureus* but had zone of inhibition (6mm) on *Salmonella* which is also regarded as resistance. According to Pate, (2017), zone of inhibition >14mm is susceptible while <14mm is resistant. Similarly, PPA was resistant to *Staph. aureus* and *Salmonella* (10mm). Ntukidem *et al*., (2020) also reported the ineffectiveness of EFBA and PPA on *Staph. aureus*, *Bacillus spp* and others which is similar to the findings of this study. The antibacterial resistance may be as a result of combustion, the high temperature during burning and carbonization may have killed their antimicrobial components (Ntukidem *et al*.,2020).The species of the test isolates as antibacterial susceptibility can be strain specific, the inoculum size, growth phase and the culture medium used are also factors that can affect antimicrobial susceptibility (Witkowska *et al*., 2013).

The anti-fungal activities of the bio-activators indicates that EFBA and PPA had no positive effect on the test isolates (Table 3). EFBA had a partial activity with percentage inhibition of 23.7% (Pundir *et al*., 2010) against A*. niger* with no inhibitory activity against *A. flavus* while PPA had 9.1% against A. *flavus* with no inhibitory activity against A*. niger*. Ntukidem *et al*.,(2020) also reported that furnace ashed EFBA and PPA did not inhibit *Aspergillus fumigatus*, which is comparable with this present study even though different strain of *Aspergillus* was used. This could be that the phytochemicals responsible for their antimicrobial inhibition were lost during ashing due to high temperature.

## 5. Conclusion

The results of this study suggests that empty palm fruit bunch ash (EFBA) and unripe plantain peel ash (PPA) possess the potential for use as alkaline bio-activator for charcoal, an alternative to the conventional chemical activators.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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