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Chemical Composition, Characterization and Antioxidant Property of a Food Additive Prepared from *Vigna mungo* (Black Gram Lentils) Plant Waste

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Abstract

Assam is a state of Northeast India and the tribal and non-tribal people here use different naturally available food materials and modify those through traditional processes for preservation and utilization for the purpose. An ethnic food additive called Khar, prepared from *Vigna mungo* (Black gram lentils) plant waste; its chemical parameters, and antioxidant properties has been studied in this work. The pH, alkalinity, hardness, metal content, and functional groups present in the ash sample and the extract are determined using titration method, atomic absorption spectrophotometer, SEM-EDX and FTIR. Some essential minerals present in the sample are K, Na, Mg, Ca, Cu, Mn, Fe, etc., and the basic property of this additive makes it a good antacid.

Keywords: Agri-Waste, Alkaline, Food Additive, Khar, *Vigna mungo*

1. Introduction

Assam is well known for its diverse cultural heritage and belongs to different tribal and non-tribal people who eat enormous indigenous food dishes. One very popular dish is called Khar which is cooked by adding a liquid alkaline food additive prepared mainly from banana tree ashes. When it is made from banana, it is called "Kolakhar" as banana is termed as "Kol" in Assamese language. It can be prepared from the ashes of banana stem, rhizome, and peel and mainly *Musa balbisiana Colla* variety is used for it[1]. Not only the banana ash extract is used as food additive, the young pseudo stem, the fruit, as well as banana flower is cooked as different type of dishes and is well known for high iron and fiber content[2]. When the pseudo stem of a banana tree is matured, it is cut into small pieces and fully dried under sunlight during October-November (dry season starts in Assam) of the year. Then the substances are burned, and the ashes are filtered to collect the smallest particle size using bamboo made sieves. It is stored well in a container for the use of the whole years. Whenever the extract is needed, people make it in small amounts and use it as edible soda and for all other purposes[1].

In Assam, India, the Bodo tribe and other non-tribal communities regularly cultivate Black gram lentil (*Vigna mungo*) plant for its use as a food dish called Daal. After separating the seeds; the plant materials are generally thrown away as waste materials which have no commercial value. The rural people usually burn these dry postharvest plants thoroughly in the open air and the ashes thus obtained are used as an insect killer in the crop fields. It is important to mention that the water extract of ash obtained from Black gram lentil (*Vigna mungo*) plant is highly alkaline in nature due to which the people of this region use it as an alternative of "Soda" or $NaHCO_3$ and used as an antacid. This water extract is locally referred to as *Khar* or *Khardwi* (in Bodo tribe language).

Since this 'Khar' is basic in nature, traditionally people use it as antacid or other digestive disorders. They use it for cleaning a freshly cut injury from bacterial attack. It can be utilized for multiple purposes in villages such as washing clothes as well as natural shampoo. Farmers use it for some diseases in cattle and to prevent the attack of

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leaches[3]. There are other uses of banana extract which have been reported for dysentery[4], pinworm infection[4], sore throat, insecticidal[5], antihelminthic[6], anti-ulcer genic, anti-microbial[7], anti-venom, anti-allergic, anti-hyperlipidemia, antidiabetic[8], anti-oxidant[9,10] as well as anticancer properties. The liquid exudates of banana trunk in treating infertility in males have also been reported[3].

B. Das and S. Bordoloi have used banana ash and carbonates of Na and K and their mixtures for the traditional methods for removal of iron from ground water[11,12]. The ashes of Banana rind, banana pseudo stem, banana leaf, rice husk, and bamboo was taken for this study applying the principle of precipitation at high pH caused by ash. Banana pseudo stem, bicarbonate of K was found to be most effective for the purpose which maintains the pH[11,12]. Thermally dried banana waste is converted into an adsorbing material by incorporation of biopolymeric matrix and used in heavy metal removal like Cr, Fe, Pb, and Zn and shows great efficiency up to 100 % removal[13]. Water Extract of Banana (WEB) is used for palladium acetate-catalyzed ligand-free Suzuki-Miyaura cross-coupling reaction which is a green method. This method offers a mild, efficient, and highly economical alternative to the existing protocols since the reaction proceeds in WEB at room temperature in air for very short reaction times (5-90 min) under 'ligand/external base/external promoter/organic media' free conditions[14].

Since the ash of banana waste *Musa balbisiana* has a variety of uses and the extract of it is beneficial as a food additive, people of Assam have used other plant-based ashes for the same purpose. For example, the plants of Black gram lentils (*Vigna mungo*), Black benni seed (*Sesamum radiatum*), Potato (*Solanum tuberson*), Coconut (*Cocos nucifera*) shell husk and Mustard plant (*Brassica nigra*) are after harvesting dried in sunlight and burnt to make the ashes. The ash extracts are used using the same procedure used for banana ash extract. The scientific study of banana ash and its extract has been done by many researchers, however the other plant ash-based materials have not been studied much yet.

In this work, Black gram lentil (*Vigna mungo*) which is popularly spoken as Mati daal or Matikalai here, ash and the extract of the ashes has been investigated to determine its physico-chemical parameters. The surfaces of the ash particles are analysed by using SEM-EDX and functional group analysis by using FTIR. This extract may have some other good properties like antioxidant behaviour, which is studied by free-radical scavenging activity of the extract using DPPH (*2,2-diphenyl-1-picryl-hydrazyl hydrate*) Sánchez-Moreno *et al*[15].

2. Materials and Methods

2.1 Materials

The native women used to prepare the ashes at home and sell it in the vegetable market so the ash was collected from them. The extract was prepared in the laboratory by following the similar procedure used by the villagers except using distilled water. A certain weight amount of ash was suspended in distilled water and stirred and kept overnight. Next day it was filtered and the filtrate was used as the extract for analytical purposes. All the chemicals required for different analysis were taken from Thermo Fischer Scientific of AR grade and used as such.

2.2 Methods and Instruments Used

The villagers don't use the exact measured amount of ash for making the extract. They use the dried half of the coconut shell where a small slit is made at the bottom (Figure 1 (vi)). Small pieces of dried bamboo sticks were inserted through the slit. The ash used to be taken from the coconut shell to below 3-4 cm from the top. Water is added to the ash making the volume to the top of the coconut shell. The water gets enough time to pass through the fine particles of the ash and it falls very slowly into the glass or other pot which is placed at the bottom of the coconut shell as shown in Figure 1(vi). Similar process was tried in the laboratory preparation of the ash extract; however, after trying different amounts of ash 1g in 100 mL water was taken as the best weight of the ash and the volume of water which gives the maximum pH.

The pH of the extract was determined by using Labtronic digital pH meter Model No. LT-11, after calibrating the instrument at pH 9.20, 7.00, and 4.00. The alkalinity was determined by double titration method using phenolphthalein and methyl orange indicator[16]. The total hardness was measured by applying the complexometric titration method using EDTA[17].

The concentration of the different metals present in the extract sample was determined by using Atomic Absorption Spectrometer (AAS) made by Thermo Scientific model no.: ICE3000 Series. FTIR of ash was studied using Perkin Elmer SPECTRUM 100 model.

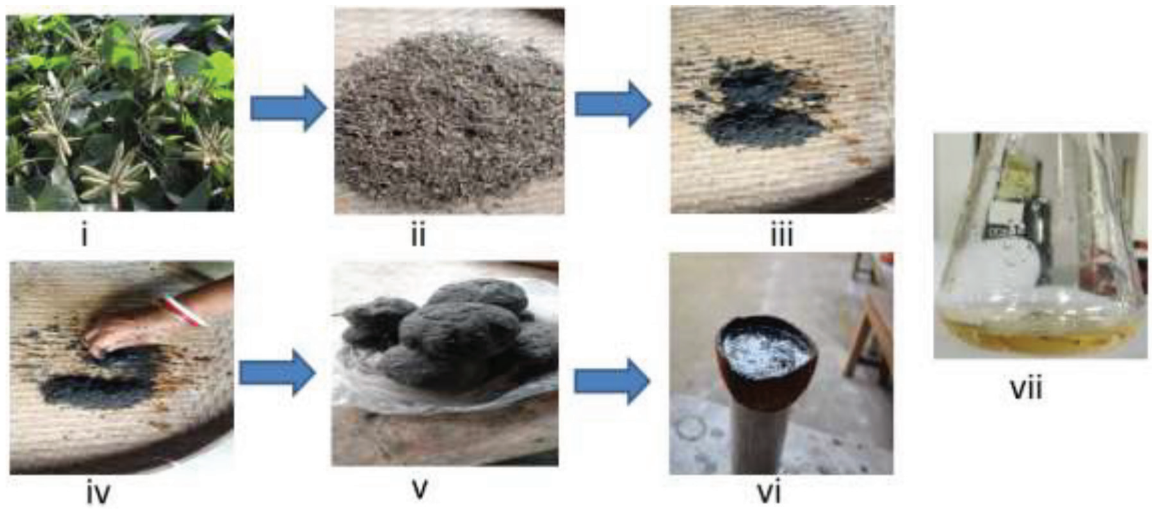

Figure 1. The process of making the *Vigna mungo* (Black gram lentils) plant waste ash extract. (i) Young Black gram lentil plant (ii) After harvesting the dried plants, (iii) burnt ash, (iv) & (v) preparation of big tablets of ash, (vi) extraction using coconut pot and glass, (vii) Laboratory prepared ash extract.

The surface morphology of the ash sample was studied by using SEM (scanning electron microscope) made by JEOL, JAPAN, model no. JSM 6390LV. The percentage of different elements was determined by EDX (Energy Dispersive X-Ray Analysis) technique.

Antioxidant activity of the ash extract was estimated by determining the free-radical scavenging activity. This was carried out using the stable radical DPPH (*2,2-diphenyl-1-picryl-hydrazyl hydrate*), as described by Sánchez-Moreno *et al*[15]. The antioxidants present in the extract scavenged the DPPH radical by donation of protons forming the reduced DPPH. This is indicated by the change in color from purple to yellow after reduction. The extent of discoloration shows the free radical scavenging abilities of the sample/antioxidants by their hydrogen donating ability. The color change is since the electrons are paired up and as a result the solution loses color stoichiometrically depending on the number of electrons taken up[18].

3. Results and Discussion

The results of different physicochemical parameters and element content of the extract of ash are presented in Table 1. Figure 2 shows the change of pH with dilution. The surface morphology of the ash has been observed from SEM micrographs (Figure 3) and the presence of different elements in the solid ash samples can be observed from the EDX data presented in Table 2. The presence of different functional groups can be observed from the FTIR spectrum in Figure 5.

Table 1. Physicochemical parameters observed from different experiments and analysis

Parameter	Amount	Element	Amount (ppm)
pH	10.94	Na	169.87
Alkalinity (Bicarbonate)	1244 ppm	K	323.25
Alkalinity (Carbonate)	1764ppm	Ca	6.29
Total Hardness	148 ppm	Mg	122.23
Chloride	177.25 ppm	Fe	0.062
TDS	1120 ppm	Mn	0.12
Conductivity	9.3 mS/cm	Cu	0.20

* Metals are detected with AAS analysis

3.1 Physicochemical Parameters

The different parameters estimated have been presented below. The pH value indicates the high basicity of the extract which can be correlated with the values of alkalinity. High carbonate and bicarbonate makes the extract highly alkaline. High value of carbonate also makes this extract for the utilization of this as a basic food additive[1]. The preparation of the extract by the villagers does not follow a proper guideline regarding the amount

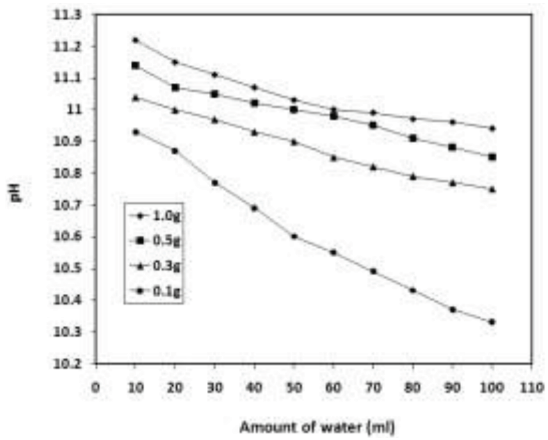

Figure 2. pH of the extract vs. the amount of water of extraction for different grams of ash.

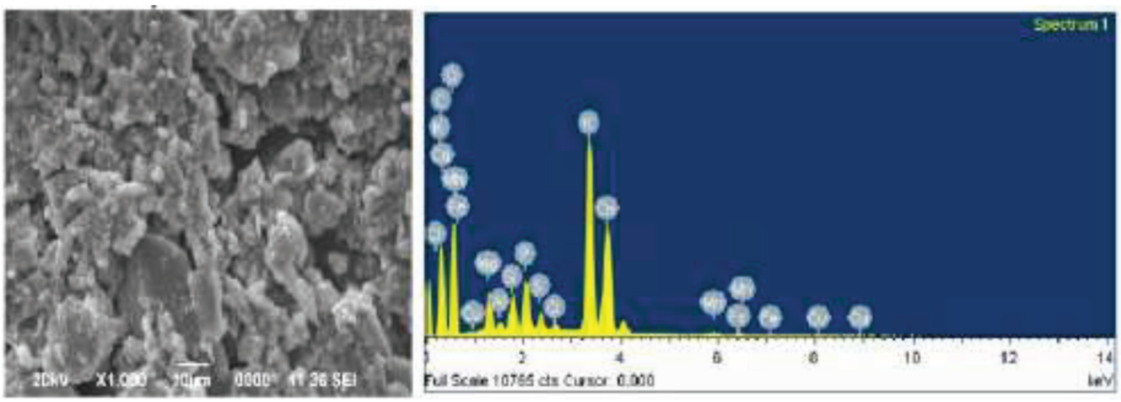

Figure 3. SEM micrographs and EDX spectrum of the ash sample showing the concentration of different elements.

of ash and water. Therefore, in this work the extract was prepared using different concentrations taking 0.1, 0.3, 0.5, and 1.0 g and per 10 to 100 mL of water and the pH of each solution was measured. The pH values show a good agreement with increase in the amount of ash and decrease with the amount of increase in water, which is obvious. The extract obtained directly from the villagers shows a pH of 10.94. Some tribal people in Assam also use this as the alternative source of common salt which may be a traditional knowledge that this liquid extract contains a lot of potassium and sodium. This resembles the value of the extract prepared by using 1 g of ash per 100 mL of water. So, this concentration of the extract was taken as the standard for future analysis.

The hardness of the extract will not affect the food prepared since only 5-10 mL of the extract is used for food making purpose, this value will further decrease in the food prepared. Conductivity of the extract indicates the presence of different ionic species like Ca^{2+}, Na^{+}, K^{+}, Cl^{-}, HCO_3^{-}, CO_3^{2-} etc.

3.2 Elemental Analysis

To examine the presence of different other metals present in the extract of *Vigna mungo* plant waste ash, the sample was analyzed using AAS. The different metals present as K > Na > Mg > Ca > Cu > Mn > Fe and Zn, Pb, Cd, Cr, As, Ni metals were not found. From this metal content of the extract, it can be mentioned that most of the necessary elements are present in it. Harmful elements like As, Cd, and Pb are not there. This extract is better in comparison to banana ash extract in terms of the presence of Pb, because as per previous work on banana ash extract, a trace amount of Pb is found in the sample[1]. SEM-EDX analysis of the *Vigna mungo* plant waste ash also has shown that there is no Pb in the sample.

Table 2. Percentage of different elements present in the ash sample analyzed by EDX

Element	Weight%	Atomic%
C K	30.44	43.15
O K	40.47	43.07
Mg K	2.15	1.51
Al K	0.35	0.22
Si K	1.66	1.01
P K	2.62	1.44
S K	0.92	0.49
Cl K	0.36	0.17
K K	12.42	5.41
Ca K	7.72	3.28
Mn K	0.32	0.10
Fe K	0.29	0.09
Cu K	0.28	0.07
Totals	100.00	

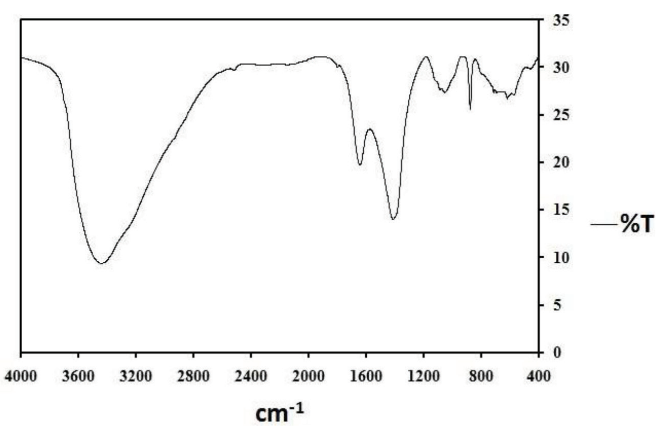

Figure 5. FTIR spectra of the ash.

3.3 SEM-EDX Analysis

The surface morphology of the *Vigna mungo* can be observed from the SEM image (Figure 3) at different resolutions. From the picture of the surface, it can be observed that it is almost homogeneous, and there is a development of agglomeration of particles of different sizes. Since the surface is uniform and the particles are also smaller in size, therefore it may have large surface area which can be used as a catalyst for some other chemical reactions[20]. This surface area may be analyzed further to check the adsorption properties of this ash. Already banana ash has been applied for the successful removal of iron from groundwater, so this ash may also be applied for the similar purpose[11,12]. The adsorption of other heavy metals may also be possible on this ash.

The EDX spectrum and the data obtained are presented in Figure 3 and Table 2. It can be observed that the percentage of different elements follows the order O > C > K > Ca > P > Mg > Si > S > Cl >Al > Mn > Fe > Cu. The presence of the highest amount of oxygen and carbon is obvious because these two are the most abundant elements present in any living body. The presence of a high amount of silicon may be attributed to the presence of clay powder in the sample. Since these plants are generally grown in sandy soils near the riverside and after harvesting, these are dried and burnt only after manually removing the clay or soil particles. Some other essential minerals like Fe, Mn, Cu, Zn which come to the water after extraction make it more beneficial as a food additive.

3.4 FTIR of Ash

In the spectrum, it is seen that the broad peak appears at 3418 cm^{-1} which indicates the presence of -OH group due to the water molecules adsorbed on the surface of the calcined *Vigna mungo*. The presence of peaks at 1646 cm^{-1}, 1416 cm^{-1} may be due to the C-O stretching vibrations[19,20]. The peaks at 1041 cm^{-1} and 875 cm^{-1} may be due to Si-O-Si bond.

The peak at 701 cm^{-1} to the K-O and Ca-O stretching vibrations which may be due to the presence of K_2O and CaO in the calcined *Vigna mungo*. In this study, the FT-IR analysis is in satisfactory agreement with the EDX data which confirmed the presence of oxides and carbonates of metals in the calcined *Vigna mungo* ash.

3.5 Antioxidant Activity

In this study, 1 mL of ash extract aliquots was mixed with 3 mL of a methanolic DPPH solution (0.1 mM). The resultant solution mixture was incubated for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The percentage of DPPH radical scavenging activity against different concentrations of ash extract (100-500 mg/mL) was then plotted to get the amount necessary to decrease the initial concentration of DPPH concentration by 50% [IC_{50}(sample)].

It has been observed that the absorbance of the reaction mixture has decreased, which indicates higher DPPH radical scavenging activity. The ability of the DPPH to scavenge the radical was calculated using the following equation.

DPPH radical scavenging activity (%)

Where AB is the absorbance of the blank, AA is the absorbance of sample.

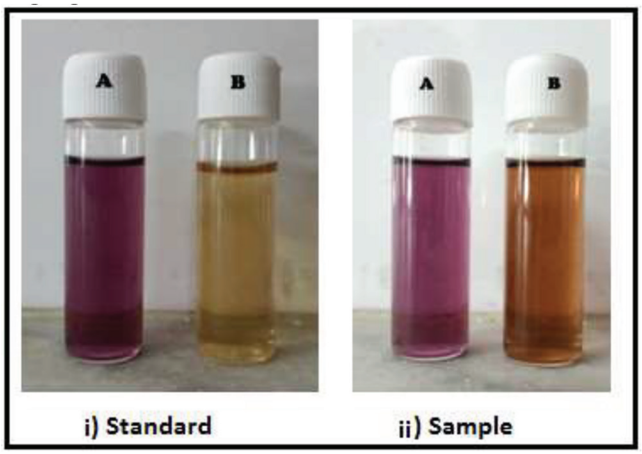

Figure 6. Representation of colour change of DPPH radical non-inhibited- Purple (A) before incubation and radical inhibited yellow (B) after 30min incubation, (i) Ascorbic acid (Standard) inhibition (ii) sample inhibition.

In this study, Ascorbic Acid was used as the standard and a calibration curve was plotted with % DPPH radical scavenging versus the concentration of standard.

The IC_{50} of the standard ascorbic acid and ash extracts was found from straight-line equation. The IC_{50} value of the ash extract was found to be 3.360 mg/mL with respect to IC_{50} value of 4.220 µg/mL of the standard. The low antioxidant activity of the ash extracted can also be physically observed by the slow rate of change in color and after incubation the color appeared to be yellowish brown. Lim Y *et al.*, have explained the low activity in these ways- some phenolic compound reacts with DPPH reversibly and slowly, which may result in low antioxidant activity[21-23]. Another possible reason is that the agro waste materials were burnt at very high temperature which could lead to the decomposition of some phenolic compounds due to which its extract is having low antioxidant activity[24].

4. Conclusions

The *Vigna mungo* plant ash and the extract of the ash are used for different purposes by the people of Assam, India; however, the extract is used mainly as food additive.

The pH of the extract is found to be 10.94 which indicates the high basicity of the sample which may be correlated with the presence of high amount of potassium present in the sample. The alkalinity of the sample may also be correlated with the presence of a high amount of

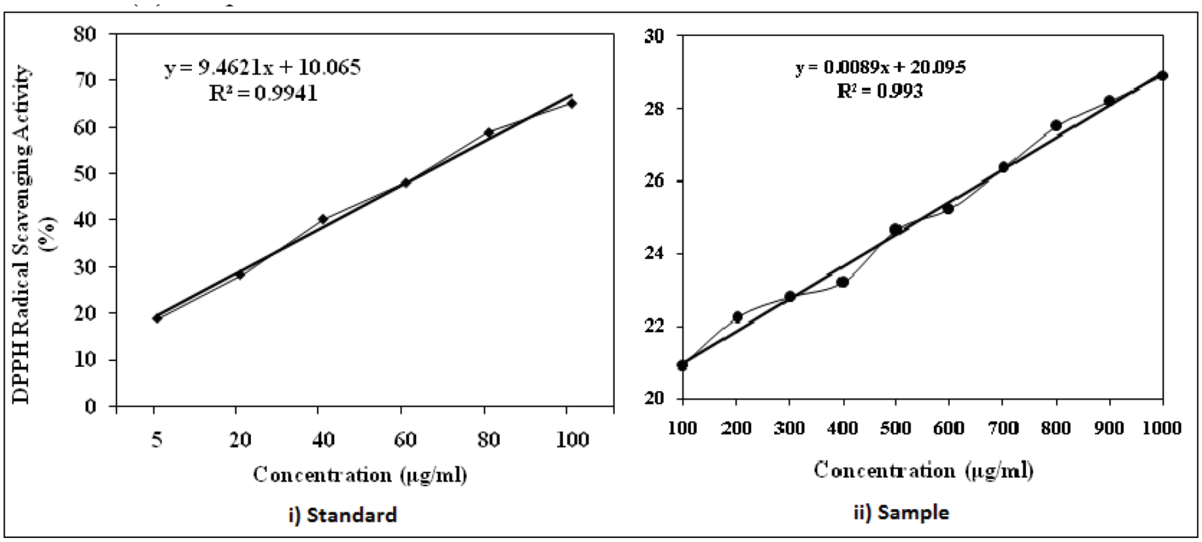

Figure 7. Percent of DPPH radical scavenging activity of (i) standard and (ii) sample

basic compounds in the solution. From the AAS data, the amount of different metals present in the sample is K>Na>Mg>Ca>Cu>Mn>Fe, other harmful elements like As, Cd, and Pb are not there. FTIR spectrum shows C-O, M-C and M-O interactions. From the surface analysis using SEM, it is observed that it is almost homogeneous, and there is a development of agglomeration of particles of different sizes. Since the surface is uniform and the particles are also smaller in size, therefore it may have large surface area which may be used as catalyst. This surface area may be analyzed further to check the adsorption properties of this ash. Already banana ash has been applied for the successful removal of iron. It can be observed that the percentage of different elements follows the order O > C > K > Ca > P > Mg > Si > S > Cl >Al > Mn > Fe > Cu. Presence of K, Na, and Ca is also obvious from the plant type. Some other essential minerals like Fe, Mn, Cu, Zn which if comes to the water after extraction makes it more beneficial as food additive. The ash extract has some free radical scavenging activity which can be understood from DPPH antioxidant activity study. This type of ash and extract of ashes have been used for different purposes in chemistry, like as a catalyst in biodiesel production, heavy metal removal, and a base for organic reactions. The *Vigna mungo* plant waste ash may also have this type of applicability in the field of chemistry.

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