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# PHYTOCHEMICAL, PROXIMATE COMPOSITION AND ANTIMICROBIAL ACTIVITIES OF N-HEXANE, CHLOROFORM AND ETHYL ACETATE EXTRACTS OF IGBO OKRA (*ABELMOSCHUS ESCULENTUS*) PODS.

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## ABSTRACT

Literature abounds to support the fact that geographical location influences the phytochemistry and proximate composition of a plant. On this premise, this current study aims to evaluate the phytochemical profile, proximate composition as well as the antimicrobial activities of the n-Hexane, Chloroform and Ethylacetate extracts of *Igbo Okra* pods planted in Ikwuano LGA, Abia State, East of Nigeria of tropical climate. Igbo Okra pods were harvested from a farm in Ikwuano LGA, Abia Sate, South Eastern Nigeria, washed and the seeds separated from the pods. The pods were air dried for two weeks in a room, pulverized using a grinding machine and stored in an airtight container for subsequent use in the study. The results were presented in tabular form after statistical analysis using SPSS statistical software and statistical significance determined using Duncan Multiple Range test at 95% confidence level. The data showed thus; Proximate analyses of Igbo Okra pods contained, 13.47mg/100g for Moisture, 10.79mg/100g for Ash, 3.85mg/100g for crude fat, 11.37mg/100g for Crude fibre, Crude protein was 1.74mg/100g and 58.78mg/100g for carbohydrate. The data for the phytochemical analyses showed the presence of Alkaloids, Terpenoids, Flavonoids, Tannins, Phenolics, Carbohydrates, Reducing Sugars and Steroids. The data for the quantitative phytochemical composition of Igbo Okra pods are as follows; Tannins 18.01mg/100g, Total Phenolics 954.30mg/100g, Steroids 1.12mg/100g, Terpenoids 36.12mg/100g, Flavonoids 111.46mg/100g, 588.89mg/100g for Alkaloids, Reducing Sugars 440.58mg/100g, Carbohydrates 1667.53mg/100g and Glycosides 14.61mg/100g. The obtained from this study results show that Igbo Okra pods possess natural products that could be of immense therapeutic value in the quest for controlling microbial resistance and treatment of diseases, giving credence to its use in ethnopharmacology.

**KEYWORDS:** Igbo Okra *Abelmoschus esculentus* (AE), Proximate Analysis, Phytochemical composition and antimicrobial activity, n-Hexane, Chloroform, Ethylacetate.

## INTRODUCTION

Ikwuano is a local government area in Abia state located in the South Eastern Nigeria. It is home to the National Root Crops Research Institute (NRCRI), Michael Okpara University. The GPS coordinates of Ikwuano are as follows, 5°25'59.99" N 7°34'0.01" E, and it lies between the latitudes 5 24lN and 5 30lN and between the longitudes of 732lE and 737lE (Chidiebere-Mark, 2018). The vegetation of Ikwuano is predominantly lowland rainforest, which makes it suitable for cultivation of a vast majority of crops; tree, shrubs and tubers. This has led to the area becoming a major supplier of food for Abia State. (Chidiebere-Mark, 2018).

"Okwuru" as it is called in Igbo language or Okra, Lady's Finger has the botanical name *Abelmoschus esculentus.* It is a vegetable touted to be rich in several phytochemicals as well as vitamins and minerals. It is a flowering plant that belongs to the family "Malvaceae" and genus "Abelmoschus". It appears as an edible green pod with a slimy interior filled with seeds arranged in a Marginal appearance. It is native to the tropical and subtropical climatic regions of which south

eastern Nigeria belongs. Dheba *et al*, 2017 reiterated the importance of the chemical composition of plants in nutrition and in treatment and management of diseases. It is common knowledge that the physical and chemical properties expressed by a plant is actually conferred by Phytochemicals (secondary metabolites) and these compounds are responsible for growth, color, taste and also confers protection against xenobiotics and foreign bodies amongst others. (Dheba *et al*, 2017)

Igbo Okra (*Abelmoschus esculentus*) plant has been used by a vast majority of tribes in traditional medicine for the treatment and management of several medical conditions and they have been documented in some notable research publications.

The presence of these chemical composition in plants is imperative in nutrition and in treatment of several diseases (Dheba *et al*., 2017).

Turkish Traditional medicine uses *Abelmoschus esculentus* seeds to reduce blood glucose levels and in management of Diabetes Mellitus.

Onakpa et al., 2013 established that *Abelmoschus esculentus* possesses diverse medicinal properties and in time past has been used for anti-microbial, antidiabetic, anticancer, analgesic, antioxidant and anti-plasmodial purposes (Onakpa *et al.*, 2013).

Traditional medicine practitioners in India use various parts of *Abelmoschus esculentus* are used in management and treatment of dysentery and diarrhea, inflammation and irritation of the stomach bowels, kidney catarrh infections, dysuria, plasma replacement and gonorrhea as well as Ulcer. (Chanchal *et al*., 2018).

Nwankwo *et al.*, 2021 showed that the plant seed possessed pharmacologically active chemicals of therapeutic value. (Nwankwo *et al*., 2021)

## MATERIALS AND METHOD

### Sample Collection, Processing and Identification

Samples of *Abelmoschus esculentus* (AE) pods were collected from a farm in Ikwuano local government, Abia state. The plant was identified and authenticated by Dr. Omosun Garuba of the department of plant science and biotechnology, Michael Okpara University of Agriculture Umudike. The samples were cut open and the seeds separated from the fruit. Both fruit and seeds were air dried, pulverized using a blender (TSK 949 West point, France) and stored in airtight plastic containers.

### Determination of Tannins

The total tannin was estimated using the Folin Ciocalteau's method (Kaur and Kapoor, 2002). 1ml of the aqueous extract of the sample was mixed with 0.5ml of Folin - ciocalteau's reagent, followed by the addition of 1ml of saturated Sodium bicarbonate solution and 8ml of distilled water. The reaction mixture was allowed to stand for 30 minutes at room temperature. This was followed by centrifugation to obtain the supernatant and its absorbance was measured at 725nm using UV-Visible spectrophotometer. Increasing concentrations of standard tannic acid was prepared and the absorbance of various tannic acid was prepared and the absorbance of various tannic acid concentrations was plotted to derive a standard graph. The tannic content was expressed as mg tannic acid equivalent per 100g of the sample.

### Determination of Total Phenol

Total phenolic compound was estimated using the folin-ciocalteau method (Kaur and Kapoor 2002). It is based on measurement of the amount of substance being tested needed to inhibit the oxidation of the reagent. 1ml of the aqueous extract was oxidized by adding to 1ml of folin-ciocalteau solution in a test tube. The reaction was neutralized with 1ml of 20% sodium carbonate. Different concentrations of sample extracts of the plant were prepared and then 100µL taken from each concentration and mixed with folin ciocalteau's reagent (1/10 dilution) and 1.5ml of $Na_2CO_3$ 3%(w/v). The blend was left to stand for 15 minutes in the dark corner at 25°C. The absorbance of the blue coloured solution of all samples was measured at 765nm. The results were expressed in mg of Gallic acid equivalent (GAE) per gram of dry weight of okra powders.

### Determination of Total Flavonoid

Determination of flavonoid content was done using the method described by Zhishen *et al.,* (1999). A 0.5ml aliquot of appropriately diluted sample solution was mixed with 2ml of distilled water and subsequently with 0.15ml of 5% $NaNO_2$ solution. 6 minutes later, 0.15ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 minutes after which 2ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5ml and then the mixture was thoroughly mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was determined at 510nm versus water blank. The analysis was performed in triplicates and the results were expressed as Rutin equivalents.

### Determination of Steroids

1g of the extract was macerated with 50ml of distilled water and filtered. To 1ml of the filtrate 2ml of cyanogen solution was added and the solution left to stand for 30 minutes. The absorbance was read at 550nm. The experiment was done according to methods described by Ekwueme *et al*., 2015.

### Determination of Glycosides

1g of the extract was macerated with 50ml of distilled water and filtered. To 1ml of the filtrate 4ml of alkaline picrate solution was added. The mixture was boiled for 5minutes and allowed to cool. The absorbance was read at 490nm.

**Determination of Reducing Sugar**
1g of the extract was macerated with 20ml of distilled water and filtered. To 1ml of the filtrate 1ml of alkaline copper reagent was added. The mixture was boiled for 5 minutes and allowed to cool. Then 1ml of phosphomolybdic acid reagent was added and 2ml of distilled water was added and the absorbance read at 420nm.

**Determination of Soluble Carbohydrate**
1g of the extract was macerated with 50ml of distilled water and filtered. To 1ml of the filtrate, aqueous solution of picric acid was added and absorbance read at 580nm.

**Determination of Alkaloids**
1g of the extract was macerated with 21ml of ethanol and 20%$H_2SO_4$(1:1v/v). 1ml of the filtrate was then added to 5ml of 60% $H_2SO_4$. After 5 minutes 5ml of 0.5% formaldehyde in 60% $H_2SO_4$ was mixed with the mixture and allowed to stand for 3 hours. The absorbance was read at 565nm.

**Determination of Terpenoids**
1g of the extract was macerated with 50ml of ethanol and filtered. To 2.5ml of the filtrate, 2.5ml of 5% aqueous phosphomolybdic acid solution was added and mixed. The mixture was left to stand for 30 minutes and then made up to 12.5ml with ethanol. The absorbance was taken at 700nm. (Ekwueme *et al*., 2015).

**PROXIMATE ANALYSIS OF IGBO OKRA PODS**
**Determination of Protein Content**
Protein content was determined using the Kjedahl method which involved digestion of the protein followed by distillation and titration.

**Digestion:** 2g of the aqueous extract was weighed into a Kjedahl flask. 5g of anhydrous sodium sulphate was added followed by the addition of 1g of copper sulphate and a speck of selenium. 25ml of concentrated sulphuric acid and 5 glass beads were introduced into the mixture. The mixture was heated in the fume cupboard rapidly at first and then increased heating with intermittent shaking till the solution became green in colour. There were black particles at the neck and mouth of the flask which was cleaned using distilled water. It was heated again gently till the green colour disappeared and then allowed to cool. After cooling, the digest was transferred with several washings into a 250ml volumetric flask and made up to the mark with distilled water.

**Protein Distillation and Titration**: Distillation ensued using the Markham's distillation apparatus (Pearson 1976). The apparatus was steamed for about 15 minutes before use. Under the condenser, 100ml conical flask containing 5ml of boric acid indicator was placed such that the condenser tip is under the liquid. 5ml of the digest was pipetted into the body of the apparatus via a small funnel aperture, the digest was washed down the distilled water followed by 5ml of 60% NaOH solution. The mixture was steamed through for about 5-7 minutes to collect enough ammonium sulphate. The receiving flask and the condensed water were removed. Titration of the solution was made in the receiving flask using 0.01N HCl and calculation of nitrogen content was made. The principle behind this method is such that protein content is measured by the amount of nitrogen.

**Determination of Crude Fibre**
An empty beaker was weighed and 3g of the sample was put into the beaker followed by the addition of 50ml of 1N $H_2SO_4$ for the hydrolysis of the protein, carbohydrate and organic element. The beaker was heated for 30 minutes. A funnel with a sieve was set on another beaker. After heating the sample was poured into the funnel and boiled water was poured on it to wash off the nutrient. The residue was put back into the beaker and 1.25% NaOH was poured onto the crude fibre to remove the acid content and boiled again for 30 minutes. Filtration ensued followed by washing again with 2000ml of boiled water to remove inorganic matter. The residue was then weighed to get the weight of the crude fibre. The loss in weight of the sample multiplied by 100 is the percentage crude fibre.

**Determination of Crude Fat**
An empty beaker was weighed and 1g of the sample was put into a conical flask. 50ml of acetone was added to remove all the nutrient in the sample and solubilize the fat. The resulting sample was filtered into previously weighed beaker. The filtrate was evaporated on a heater and allowed to cool. The beaker was then weighed again. The calculation was done using the formulae;
%Crude fat =W3-W2 /W1*100/1, where
W3= weight of beaker and oil
W1= weight of beaker
W2= weight of beaker after heating

**Determination of Moisture Content**
The sample was put in a previously weighed moisture can and oven dried for three hours at 105°C. The sample was then weighed again until the weight value was constant. The dried sample was then placed in a desiccator.

**CALCULATION**
% Moisture content = W2-W3/ W2-W1 where,
W1= weight of the moisture can.
W2= weight of the moisture can + sample.
W3= weight of the moisture can plus dried sample.
% Dry matter =100- % moisture.

**Determination of Ash Content**
**6**5g of the sample was weighed into a crucible dish followed by heating of the crucible on a covered heater. It was left for 24 hours to ash completely at a low temperature after which forceps were used to remove the crucible from the heater.

**Calculation**
% Ash (dry weight) = weight of ash/ weight of original sample*100/1.

**Determination of Carbohydrate Content**
The total carbohydrate content was determined by subtracting the percentages of the other food content from 100%.

**Calculation**
%Carbohydrate = 100% - (moisture + ash + crude fibre + crude fat + crude protein)

**Extract Preparations**
Hexane (technical grade, BDH) was used as an extractant in the assays using a ratio of 1:10 of okra peel/seed powder to extractant. The extraction procedure developed and described by Eloff (2001) was used 30g of each plant materials was extracted with 300ml hexane. The mixture was shaken at high speed for 10minutes in a shaking machine. The extracts were then centrifuged at 6000 rpm for 10minutes. After centrifugation, the supernatants were filtered through Whatman No 1 filter paper and transferred into pre-weighed labelled glass vials. The solvent was removed under a stream of air at room temperature for antibacterial assay at a concentration of 10mg/ml.

**Antibacterial activity**
The antibacterial activity on (n-hexane) fractions was done using the inhibitory zone diameter (IZD), minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) (Eloff, 1998). Eight enteric pathogenic bacteria, namely; *Shigella flexneri*, *Klebsiella pneumonia*, *Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Citrobacter feudini* were used. Gentamycin and distilled water were used as controls for the bacteria. All test bacterial strains were clinical isolates gotten from the Microbiology Unit, Medical Laboratory Department, Federal Medical Centre, Umuahia, Abia State. These were identified by various biochemical tests and preserved in agar slants kept at $4^0$C.The cultures of bacterial strains were prepared by incubating at $37^0$C for 24h. the suspensions of bacterial strains with cell density of 1 x $10^8$cfu/mL was prepared by comparing with McFarland standard No 2 and was later diluted to a cell density of 1 x $10^6$cfu/ml through UV spectrophotometers at 625nm. (Sadiq *et al.*, 2016).

**Statistical Analysis**
SPSS (Statistical Package for Social Science Students) software Version 22.0 was used to analyse the data obtained from the studies and the results presented as Mean ± SD (standard deviation) in the tables displayed. The analysis of variance (ANOVA) for multiple comparisons was also used to detect significant difference where $p<0.05$ was set to indicate statistical significance using Duncan post hoc test. All results were presented in tables.

**RESULTS FOR PROXIMATE ANALYSIS OF IGBBO OKRA PODS (ABELMOSCHUS ESCULENTUS)**

| PROXIMATE PARAMETRES | PODS (%) |
|---|---|
| MOISTURE CONTENT | 13.47±0.00 |
| ASH CONTENT | 10.79±0.00 |
| CRUDE FAT | 3.85±0.00 |
| CRUDE FIBRE | 11.37±0.00 |
| CRUDE PROTEIN | 1.74±0.00 |
| CARBOHYDRATE | 58.78±0.00 |

Results are recorded in mean percentage per grams ± standard deviation

**RESULTS FOR PRELIMINARY QUALITATIVE PHYTOCHEMICAL ANALYSIS OF IGBO OKRA PODS**

| PHYTOCHEMICALS | PODS |
|---|---|
| ALKALOIDS | ++ |
| FLAVONOIDS | + |
| TANNINS | + |
| TOTAL PHENOLICS | + |
| STEROIDS | ++ |
| TERPENOIDS | ++ |
| CARBOHYDRATES | + |
| GLYCOSIDES | ND |
| REDUCING SUGAR | +++ |
| SAPONINS | ND |

**RESULTS FOR PHYTOCHEMICAL COMPOSITION OF IGBO OKRA FRUIT**

| PHYTOCHEMICALS | PODS (Mg/100) |
|---|---|
| TANNINS | 15.30±0.61[f] |
| TOTAL PHENOLICS | 954.30±31.00[b] |
| STEROIDS | 1.12±0.01[f] |
| TERPENOIDS | 36.12±2.12[f] |
| FLAVONOIDS | 111.46±1.87[e] |
| ALKALOIDS | 588.89±29.73[c] |
| REDUCING SUGAR | 440.58±12.46[d] |
| CARBOHYDRATE | 1667.53±5.13[a] |
| GLYCOSIDE | 14.61±0.25[f] |

Results are recorded in mean percentage per 100 grams ± standard deviation

**RESULTS FOR THE ANTIBACTERIAL ACTIVITIES METHANOL EXTRACTS OF IGBO OKRA PODS**

| | 100% | | 50% | | | MIC (%) | | | MBC (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Isolates** | **N*** | **C*** | **E*** | **N*** | **C*** | **E*** | **N*** | **C*** | **E*** | **N*** | **C*** | **N** |
| Shigella spp | 12.3 | 14.3 | 11.7 | 13 | 10 | 9.7 | 25 | 25 | 50 | 50 | 50 | 100 |
| Klebsiella spp | 11.3 | 15.3 | 15.0 | 9.0 | 9.7 | 7.7 | 25 | 50 | 50 | 50 | 50 | 100 |
| Escherichia spp | 11.0 | 11.0 | 15.0 | 6.3 | 5.0 | 9.7 | 50 | 50 | 25 | 100 | 100 | 50 |
| Staphylococcus spp | 10.3 | 7.7 | 9.7 | 6.7 | 3.7 | 5.3 | 50 | 50 | 25 | 100 | 100 | 50 |
| Enterobacter spp | 6.7 | 5.3 | 4.7 | 3.0 | 2.3 | 100 | 25 | 12.5 | 200 | 50 | 25 | |
| Psendomas spp | 4.7 | 5.0 | 11.3 | 4.0 | 1.3 | 57 | 100 | 25 | 25 | 200 | 50 | 100 |
| Proteus spp | 13.7 | 7.7 | 107 | 7.0 | 3.7 | 57 | 50 | 12.5 | 12.5 | 100 | 25 | 25 |
| Citrobacter spp. | 6.7 | 77 | 10.7 | 2.3 | 4.3 | 6.3 | 12.5 | 12.5 | 12.5 | 22 | 25 | 25 |

**N* n-Hexane**
**C* Chloroform**
**E* Ethylacetate**

## DISCUSSION

Plants being rife with phytochemicals of diverse kinds is of benefit to humans and animals that consume them because there is a chance of therapeutic benefits as well as toxicity and this study investigated Igbo Okra pods as a source of useful phytochemicals that could further be of benefit in controlling the growth of microbes as an antibiotic. Nwankwo *et al*., 2021 in their previous study had stated the phytochemical and proximate composition of Igbo Okra seeds and this spurred the inquest into the pods of the same plant. The results from this current study showed the abundance of therapeutically active phytochemicals in the plant which gives credence its use in traditional medicine.

The presence of polyphenolic compounds (Bors et al., 2002) and Flavonoids (Duthie and Dubson, 1999) in the plant suggests that it possesses potent antioxidant potentials, being able to quench harmful free radicals in the in vitro and in vivo. The antimicrobial potentials of Igbo okra stems from its abundant phenolic compound (Shehadi et al., 2014). Flavonoids, have been shown in studies to possess bacteriocidal and bacteriostatic activities. This is not a surprise as they are produced to defend the cell against foreign bodies. Being lipophilic there is a tendency that their antimicrobial activities stem from them being able to disrupt the Bacterial cell wall. (Cowan et al., 1999). The results for the proximate analysis show that Igbo Okra seeds could be of nutraceutical benefits especially in rural areas with nutritional deficiencies as it can easily be cultivated. The high carbohydrate and protein content makes it very important in the management of Protein Energy under nutrition endemic in the rural villages where this study was done. This study is all about improving the lives of the rural communities and as such it is of import to adequately justify the use of Igbo okra seeds in ethnomedicine and also as a nutraceutical, given that not all can have access to balanced diet.

In conclusion, Igbo okra pods are a good source of antioxidants in phenolics and flavonoids, also alkaloids in the control of microbial invasion, thus further studies are required to give accurately the chemical compounds responsible for these actions and possibly their mechanisms of action.

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