World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

PHYTOCHEMICAL AND PROXIMATE COMPOSITION OF IGBO OKRA (*ABELMOSCHUS ESCULENTUS*) SEEDS

Chinedum Ifeanyi Nwankwo[1]*, Njoku Juliet Chinomso[1], Nwakanma Sunday Ndubuisi[3], Ajah Obinna[1], Alaebo Prince Ogochukwu[1], Uhuo Emmanuel Nnaemeka[1], Pipi Okechukwu Gift[2], Ochiabuto Esther C.[1] and Umeh Virginus A.[1]

[1]Department of Biochemistry, Michael Okpara University of Agriculture, Umudike.
[2]Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike.
[3]Federal College of Agriculture Ishiagu, Ebonyi State. Department of Science Laboratory Technology.

Corresponding Author: Chinedum Ifeanyi Nwankwo
Department of Biochemistry, Michael Okpara University of Agriculture, Umudike.

Article Received on 01/04/2021 **Article Revised on 21/04/2021** **Article Accepted on 10/05/2021**

ABSTRACT

It has been established that the geographical location of a plant has modulatory effects on the expression of phytochemicals in the plant. This idea spurred the aim of this current study, which is to evaluate the phytochemical profile and proximate composition of *Abelmoschus esculentus* (AE) seeds planted in, Ikwuano LGA, Abia State, Southeastern Nigeria which has a tropical climatic condition. Immature AE pods were harvested from a farm in Ikwuano LGA, Abia Sate, South Eastern Nigeria, washed and the seeds separated from the pods. The seeds were allowed to air dry for two weeks, pulverized to fine powder using a milling machine and used for the different analyses using standard procedures. The results obtained were statistically analyzed using the SPSS software (version 22.0) and results were presented as Mean ± SD (Standard Deviation) and statistical significance determined using Duncan Multiple Range test. The results obtained showed that for the proximate composition analyses, Igbo Okra seeds contained 10.48mg/100g Moisture, 5.21mg/100g Ash, 15.30mg/100g crude fat, 2.50mg/100g Crude fibre, 28.92 mg/100g Crude protein and 47.60mg/100g carbohydrate. The results for the phytochemical analysis showed the presence of Alkaloids, Terpenoids, Glycosides, Flavonoids, Tannins, Phenolics, Carbohydrates, reducing sugars, Steroids and saponins. The quantitative phytochemical composition of Igbo Okra seeds is as follows; Tannins 18.01mg/100g, Total Phenolics 139.88mg/100g, Steroids 0.15mg/100g, Terpenoids 43.68mg/100g, Flavonoids 164.65mg/100g, Reducing Sugars 301.36mg/100g, Carbohydrates 1172.16mg/100g and Glycosides 13.75mg/100g. Igbo Okra seeds in South East are rife with important phytochemicals which could be of pharmacological importance.

KEYWORDS: Igbo Okra *Abelmoschus esculentus* (AE), Proximate composition and Phytochemical composition.

INTRODUCTION

The Igbo people, often natively referred to as *Ńdị́ Ìgbò* are an ethnic group indigenous to the present day south eastern and South Central Nigeria as well as Equatorial Guinea (Igbodefender.com, 2020). Geographically, the Igbo homeland is divided into two unequal sections by the River Niger, an Eastern section which is large and a smaller southern section (Katherine, 2016). The vegetation in the South Eastern Nigeria is deciduous and the climate is tropical, making it home to a vast diversity of crops, trees and shrubs. Annual average rainfall of South Eastern Nigeria is at 1744 mm with bi-modal double peaks in July and September as in most parts of Southern Nigeria., while the average annual temperature between 26 and 27°C (Ngene *et al*., 2018).

Abelmoschus esculentus called *Okwuru* by *Ńdị́ Ìgbò* and Lady's Finger or okra in English is a flowering plant of the "Malvaceae" family and the genus " Abelmoschus" possessing edible green pods with a slimy interior filled with seeds. It is native to the tropical and subtropical climatic areas of which south eastern Nigeria belongs. *Ńdị́ Ìgbò* have used traditionally as a stomachic and astringent. They use it to treat Urinary tract infections, gonorrhea, diarrhea and dysentery. (Onakpa *et al.*, 2013). The whole immature plant is also edible, used to prepare a delicacy called *Òfe okwuru.* In Latin America ethnomedicine, the seeds and the leaves are used to treat tumour. In Nicaragua, the roots prepared by infusion are used to treat syphilis and stomach ulcer, diabetes, jaundice respectively and is also used as laxatives, in Nepali ethnomedicine the juice of the root is used to treat cuts, wounds and boils (Chanchal *et al.*,2018). In Unani

ethnomedicine the seeds are used to treat stomatitis, itch, dyspepsia, leukoderma, urinary discharge, gonorrhea and to quell thirst. (Onakpa,2013). It has been reported for use as antivenom and plays hepatoprotective roles (Saravanan *et al.*,2013). The use of AE in traditional medicine in several parts of the world could point to the presence of medicinal compounds present in the plant and this justifies the aim of this current study in evaluation of the phytochemical and proximate composition of AE seeds.

Location of the Igbo in the map of Nigeria in green
Source: Ngene *et al.*, 2018

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 measuring the amount of substances 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 A.E SEEDS

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
65g 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)

Statistical Analysis

The results obtained were statistically analyzed using the SPSS software (version 22.0) and results were presented as Mean±SEM (standard error of mean). The sample homogeneity test and 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. The proximate analysis and the phytochemical analysis results were presented in tables and graphs.

RESULTS

The phytochemical components of plants are responsible for their therapeutic activities (Dheba *et al.,* 2017). Proximate analysis gives insight on the relationship between the various parameters and recommended daily intake. Below are the results of the phytochemical and proximate analysis carried out on Igbo AE seeds.

Table 1: Results for Quantitative Analysis of *AE* Seeds.

Phytochemicals	Igbo okra seeds (mg/100)
Tannins	18.01±0.02
Total phenolics	1319.88±21.10
Steroids	0.15±0.01
Terpenoids	43.68±21.95
Flavonoids	164.65±2.12
Alkaloids	620.59±5.54
Reducing sugar	301.36±16.15
Carbohydrate	1172.16±17.85
Glycoside	13.75±0.19

Table 1: Values are represented as mean values ± standard error of mean of three replicates

Table 2: Proximate Composition Values of Igbo Seeds (%).

Proximate	Seed (%)
Moisture content	10.48±0.00
Ash content	5.21±0.00
Crude fat	15.30±0.00
Crude fibre	2.50±0.00
Crude protein	18.91±0.00
Carbohydrate	47.60±0.00

Results are recorded in mean percentage per grams ± standard error of mean

DISCUSSION

The results from this current study showed the abundance of therapeutically active phytochemicals in the plant which would justify its use of the plant in ethnomedicine.

It is well established that the location of cultivation of any plant has a strong influence on the expression of nutrients as well chemicals in the plant due to a number of factors ranging from, temperature, water, manure, fertilizer, herbicides and other biotic or abiotic factors peculiar to that clime. Therefore, the expression of several phytochemicals in Igbo Okra could suggest that the climatic conditions of the environment was conducive for the growth of the plant.

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.

CONCLUSION

In conclusion, the seeds of Igbo okra cultivated in Ikwuano, Abia State, Southeastern Nigeria is rich in phytochemicals beneficial to health in terms of both therapeutic and nutritional benefits. Therefore, eat okra and be well.

REFERENCES

1. Bors W, Michel C. Chemistry of the Antioxidant. Effect of Polyphenols. Ann NY Acad Sci., 2002; 957: 57–69.
2. Kaur C and Kapoor H.C. Anti-oxidant activity and total phenolic content of some Asian vegetables Int. J. Food Sci. Technol., 2002; 37: 153-161
3. Chanchal, k., Alok, S., Kumar, M., Bijauliya, K., Rashi, S., Gupta, S. A brief review on Abelmoschus esculentus. Linn. Okra. *International journal of pharmaceutical science and research,* 2018; 7(1): 58-66.
4. Cowan M. Plant Products as Antimicrobial Agents Clin. Microbiol. Rev., 1999; 12(4): 564

5. Duthie, GG, Duthie, SJ, Kyle, JAM. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutrition Research Reviews, 2000; 13: 79–106.
6. Ekwueme FN, Nwodo O FC, Joshua P E, Nkwocha C, Eluka PE. Qualitative and Quantitative phytochemical screening of the aqueous leaf extract of Senna mimosoides: Its effect in in vivo leukocyte mobilization induced by inflammatory stimulus. International Journal of Current Microbiology and Applied Sciences, 2015; 4(5): 1176-1188.
7. Ngene Ben Uchechukwu, Jonah C. Agunwamba, Imokhai T. Tenebe and Gideon O. Bamigboye (2018). Geography of Udi Cuesta Contribution to Hydro- Meteorological Pattern of the South Eastern Nigeria, Engineering and Mathematical Topics in Rainfall, Theodore V Hromadka II and Prasada Rao, IntechOpen, DOI: 10.5772/intechopen.72867.
8. Onakpa, M. Ethnomedicinal, phytochemical, Pharmacological profile of genus Abelmoschus. *Photo pharmacology*, 2013; 4(3): 643-663.
9. Saravanan, S., Pandikumar, P., Pazhanivel, N., Paulraj, M., Ignacimuthu, S. Hepatoprotective role of Abelmoschus esculentus (Linn). Moench., on carbon tetrachloride- induced liver injury. *Toxicological mechanisms and methods*, 2013; 23(7): 528: 536.
10. Zhishen J, Mengcheng T and Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 1999; 64: 555-559.