RICINUS COMMUNIS SEEDS (OGIRI-OKPEI) AND ITS PHYTOCHEMICAL COMPOSITION

[1*]Ezenobi, U. V
Dept. of Chemistry
Michael Okpara University of Agriculture
Umudike, Nigeria
E-mail: amakufj2006@yahoo.com

[2]Amaku, F. J and [3]Agbidi chioma
Dept. of Chemistry
Michael Okpara University of Agriculture
Umudike, Nigeria
E-mail: amakufj2006@yahoo.com

ABSTRACT The numerous health benefits associated with *Ricinus Communis* seeds (Ogiri-okpei) are as a result of the numerous vitamins identified and phytochemical content of Ogiri-okpei. Proximate, minerals and vitamin composition of *Ricinus Communis* were investigated using standard analysis. Result shows that the spices *Ricinus Communis* seeds contains the following phytochemicals respectively; Phytate 4.36±0.06, Tannins 0. 64±0.02 , Saponins 0.34±0.02, Flavonoid 0.13±0.01, Alkaloid 0.84±0.00, Phenol 0.08±0.00, Steroid 0.08±0.00. Other important minerals like calcium, magnesium, sodium, phosphorus and iron were present as well as vitamins (niacin, thiamin, riboflavin, ascorbic acid and vitamin A).

Keywords: *Ricinus Communis, phytate,* Steroid, niacin, thiamine

INTRODUCTION

Ricinus Communis seeds (Ogiri-okpei) is a food flavouring, produced from fermented oil seeds. It has a very strong aromatic smell that sets the whole house on a high itch once the ogiri-okpei jumps in the soup pot, [1]. Ogiri-okpei originated from West Africa, precisely Igbo part of Nigeria and it is characteristically dark-brown in appearance. [2] stated that ogiri okpei has remained the pride of culinary purposes from time immemorial and it is interesting to mention that this spice had continues to play a major role in serving as a nutritive protein substitute (being produced from leguminous seed) as well as serving an aromatic flavouring for some dishes especially soups. According to available research the term phytochemicals is a broad name for wide variety of compounds produced by plants, they are found in fruits, vegetables, beans, grains and other plants thus the following phytochemicals have been identified in ogiri-okpei spice, they include; alkaloids, flavonoids, phytates, phenols, steroids, saponin, and tanins. Those phytochemicals are the reason behind the Health functions of ogiri-okpei to humans. The ethno-medicinal uses of ogiri-okpei include: It serves as a nutritive protein substitute, it serves as an aromatic flavoring for dishes especially soups and helps as a reliable source of vitamins and minerals necessary for good health.

MATERIALS AND METHODS

The materials used are ogiri-okpei, weighing balance, oven, and beakers, moisture can, measuring cylinder, incinerator, test-tubes, test-tube holder, water bath, dropper, flat bottomed flasks, stirrer, funnel, crucible, funnel, spectrophometer, volumetric flask, desiccator, fume cupboard, filter paper, kjeldahl distiller and flame photometer.

Determination of Moisture

10 g of each sample was measured into a pre-weighed moisture can. The sample in the can was dried in the oven at 105 ^{o}C for 3 hours. It was cooled in a desiccator and weighed. It was returned to the oven for further drying after which it was left to cool and weighed repeatedly for an hour intervals until a constant weight was

obtained. The weight of the moisture lost was calculated as a percentage of weight of sample analysed. It was given by the expression [3].

$$\% \text{ moisture content} = \frac{100}{1} \times \frac{W_2 - W_3}{W_2 - W_1}$$

Where w_1 = weight of empty moisture

W_2 = weight of moisture can and sample before drying

W_3= weight of moisture can and sample dried on constant weight.

Note: the same procedure above was used for determination of moisture content for ogiri okpei and uziza seed spices.

Determination of Ash of Content

This was done by furnace incineration according to the America Organization of Analytical Chemist method. 3 g of the processed sample (ogiri okpei) was poured into a previously weighed porcelain crucible. The sample was burnt to ashes in a muffle furnace in a desiccator and weighed. The weight of the ash was expressed in percentage of weight of sample analysed as shown below:

$$\% \text{ Ash} = \frac{100}{1} \times \frac{W_1 - W_2}{weight\ of\ sample}$$

Where, W_1 = weight of empty crucible

W_2 = weight of crucible + ash

Determination of Crude Fibre Content

This is done by the America Organization of Analytical Chemist AOAC method. 3 g of each processed samples was boiled in 150 ml of 1.25% H_2SO_4 solution for 30 min under reflux. The boiled sample was washed in several portions of hot water using a two-fold muslin cloth to trap the particles which were returned back to the flask and boiled again in 150 ml of 1.25% NAOH for another 30 min under the same condition. After washing in several portions of hot water, the samples was allowed to drain dry before being transferred to a weighed crucible where it was dried in an oven at 105 ^{0}C to a constant weight. It was burnt to ashes in a muffle furnace. The weight of fibre was calculated as a percentage of weight of sample analysed.

Determination of Crude Protein

This was done by kjeldahl method. The total N_2 was determined and multiplied with factor 6.25 to obtain the protein content. 1.0 g of processed sample was mixed with 10 ml of concentrated H_2SO_4 in a digestion flask. A tablet of selenium catalyst was added to it before it was heated in a fume cupboard until a clear solution was obtained (i.e. the digest) which was diluted to 100 ml of the digest was mixed with equal volume of 45% NaOH solution in a kjeldahl distillation apparatus. The mixture was distilled into 10 ml of 4% buric acid containing 3 drops of mixed indicator (methyl red). A total of 5 ml of distilleries was collected and titrated against 0.02 M EDTA from green to deep red end point. The N_2 content and hence the protein content was calculated using the formular below:

$$\% \text{ protein} = \%N_2 \times 6.25$$

$$\%N_2 = \left(\frac{100}{w} \times \frac{N \times 14}{1000} \times \frac{Vt}{Va}\right) T - B$$

W= weight of sample

N= normality of titrant (0.02) H_2SO_4

Vt= total digest volume (100 ml)

Va= volume of digest analysed (10 ml)

T= titre value of sample.

B= titre value of blank

Determination of Phosphorus Content

This was determined by the molybdo vanadate method. A measured volume of dry ash digest (2 mg) of samples was dispersed into a 50 ml volumetric flask. The same volume of distilled water and standard phosphorus solution were measured into different flask to serve as a regent blank and standard respectively. 2 ml of phosphorus colour reagent (molybdo vanadate solution) was added to each of the flasks and allowed to stand at room temperature for 15 min. The content of the flask was diluted to the 50 ml mark with distilled water and its absorbance was measured in

spectrophotometer at a wavelength of 540 nm with the regent blank at zero.

The phosphorus content was calculated, using the formula;

$$\text{P mg/100 g} = \frac{100}{w} \times \frac{Au}{As} \times C \times \frac{Vt}{Va}$$

Where, W= weight of ashed sample

Au= absorbance of test sample

C = concentration of standard phosphorus solution

Vt = total volume of extract

Va= volume of extract analysed

Determination of Potassium and Sodium Content.

This was done using the flame photometry method. Jaway digital flame photometer was set up according to the manufacturer's instruction. It was switched on and allowed about to 10 to 15 minutes to equilibrate. Standard sodium and potassium solution were prepared separately and diluted in series to contain 10, 8, 6, 4, and 2 g of sodium and potassium respectively.

After equilibrating the instrument, 1 ml of each standard was aspirated into it and sprayed over the non-luminous flame. The optical density of the resulting emission from each standard solution was recorded. Before filtrating, the appropriate element (Na and K) was put in place with the standard course which was used to extrapolate the content of each test element and calculated as shown below;

$$\text{Na or K (mg/ 100 g)} = \frac{x}{1000} \times \frac{Vt}{Va} \times D \times \frac{100}{w}$$

Where;

X = concentration of test elements from the curve.

Concentration of Calcium and Magnesium Content.

This was done by using the versanate EDTA titrimetric method. 20 ml portion of the extract was dispersed into a conical flask and treated with pinches of making agents (hydroxylamine hydrochloride, sodium potassium ferrocyanide). The flask was shaken and the mixture dissolve. 20 ml of ammonia buffer was added to it to raise the pH to 10.00 (a point at which calcium and magnesium forms complexes with EDTA solution using crochorme black-t as indicator. A regent blank was also done from deep red to permanent blue end point. The titration value represents both Ca^{2+} and Mg^{2+} in the test sample.

A repeated sample was done to determine Ca^{2+} alone in the test sample. This was done in similarity with the above titration. However, 10% NaOH was used in place of crochrome black-T. From the above values obtained, the Ca^{2+} and Mg^{2+} content were calculated as follows.

$$\text{Ca/Mg (mg/100 g)} = \frac{100}{w} \times T - B\left(N \times \frac{Ca}{Mg}\right)\frac{Vt}{Va}$$

Where;

W= weight of sample

T= titre value of sample

B = titre value of blank

Ca= calcium equivalent

Mg = magnesium equivalent

N= normality of titrant (0.02 N EDTA).

Test for the Presence of Alkaloid

5 g of each sample was weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 hours. This was the filtered and extract was concentrated using a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and then filtered. The precipitate was dried and weighed [4-5]. The alkaloid content was calculated in percentage;

$$\%\ alkaloid = \frac{Weight\ of\ residue}{weight\ of\ sample} \times \frac{100}{1}$$

Test for the Presence of Saponin.

20 g of each of the samples was dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55 ^{o}C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water-bath at about 90 ^{o}C. The concentrate was transferred into 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was discarded. The purified process was repeated. 60 ml of n-butanol was added. The combined n-butanol extract were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water-bath. After evaporation, the samples were dried in the oven to a constant weight.

$$\%\ saponin = \frac{Weight\ of\ residue}{weight\ of\ sample} \times \frac{100}{1}$$

Test for the Presence of Tannin

0.5 g of the sample was extracted with distilled water. It was shaken for 30 minutes at room temperature and filtered. A standard tannic acid solution was prepared. 2 ml of the standard solution and equal volume of distilled water were dispersed into a separate 50 ml volumetric flask to serve as standard and regent blank respectively. Then 2 ml of each of the samples extract was mixed with 35 ml distilled water in 50 ml volumetric flask. 1.0 ml of the Folin-Denis regent was added followed by addition of 2.5 ml of saturated Na_2CO_3 solution and then distilled water. Shake the mixture, incubate at room temperature for 90 min. Take the absorbance at 760 nm with spectrophotometer.

$$\%\ tannin = \frac{Au}{As} \times\ c\ \times \frac{100}{w} \times \frac{Vf}{Va}$$

Where,

Au = absorbance of the test sample

As = absorbance of blank

C = concentration of the standard tannin solution

Vt = total volume of extract

Va = volume of extract analyzed

W = weight of the sample used

Determination of Phenol

0.2 g of the sample was dissolved in methanol and filtered to extract phenol. 1 ml of the filtrate was mixed with 1ml of folin-ciecateau regent and 2 ml of 20% Na_2CO_3 solution was added. The intensity of the developed colour was measured using spectrophotometer at 560 nm. The standard phenol was treated the same way.

$$Phenol\ content = \frac{Au - Ab\ X\ C\ X\ D}{As - Ab} \times \frac{100}{1}$$

Where;

Au = absorbance of the test sample

Ab = absorbance of blank

As = absorbance of standard phenolic solution

C = concentration of standard phenolic solution

D = dilution factor if any

Test for Flavonoid

10 g of sample was extracted, repeated with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through No 42 whatmann filter paper. The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

$$\%\ \text{Flavonoid} = \frac{weight\ of\ residue}{weight\ of\ sample} \times \frac{100}{1}$$

Test for steroid

5 g of each spice was hydrolysed by boiling in 50 ml hydrochloric acid solution for about 30 minutes. It was filtered paper, the filtrate was transferred to the separating funnel. Equal volume of ethyl acetate was added to it, mixed well and allowed to separate in two layers was discharged. The extract was dried at 100 ^{o}C for 5 minutes in a steam bath. It was then heated with concentrated amyl alcohol to extract the steroid. The mixture becomes turbid and a reweighed Whatmann filter paper was used to filter the mixture properly. The dry extract was then cooled in a desiccator and weighed. The process

was repeated two times and an average was obtained.

The concentration of steroid was determined and expressed as a percentage thus

$$\% \text{ steroid} = \frac{w2-w3}{wt\ of\ sample} \times \frac{100}{1}$$

Where,

W1 = weight of filter paper

W_2= weight of paper + steroid

Test for Phytate

The plant material was extracted with 0.2 M HCl such that we have (3-30 μg m^{-1} phytate solution). 0.5 ml of extract was pipette into a test tube fitted with a ground glass stopper. 1 ml of ferric solution was added, the tube covered with the stopper and fix if with a clip. The tube was heated in a boiling water bath for 30 min. Care was taken to ensure that the first 5 min that the tube remains well stopped. After cooling in ice water for 15 min, it was allow to adjust to room temperature. Once the tubes have reached room temperature, the content of the tube was mixed and centrifuge for 30 min at 3000 g. 1 ml of the supernatant was transferred to another test tube and added to 5 ml of 2,2-Bipyridine solution. The absorbance was measured at 519 nm against distilled water. Then calibration with the reference solutions as a substitute for the sample solution with each set of analysis was made, then the absorbance of the test sample is used to obtain the concentration from the calibration curve.

DISCUSSIONS

Table 4.1 shows the important phytochemicals of *Ricinus communis* seeds which include alkaloids, saponins, flavonoids, phenols, steroids, phytates and tannins. Some of the phytochemicals have established medicinal values [6-7] while the others phytates and oxalates are considered to be anti-nutrients [8]. Tannins, proline-rich proteins, help to inhibit the absorption of iron when present in the gastro-intestinal lumen [9]. However, the steroids content of *Ricinus communis* seeds was found to be 0.08 ± 0.00%, alkaloids content as 0.08 ± 0.00%. Alkaloids can elucidate a wide range of physiological activities in the body when consumed and are therefore widely applied in medicine. The percentage flavonoid in the seeds of *Ricinus communis* was found to be 0.13 ± 0.01% which is agreement with [10-12]. 0.34 ± 0.02% of saponin was estimated in Ricinus *communis*, this property gives them the ability to foam [13].The proximate compositions of *Ricinus communis* (ogiri-okpei) is shown in Table 4.2. The moisture mean value as 35.77 ± 0.10, Ether extract (5.62 ± 0.00), Ash content (7.71 ± 0.13), crude protein Dry (7.66 ± 0.25), and Crude fibre as 1.85±0.00.

Table 4.1: Phytochemical composition Risicnius *comminius* seeds

Phytochemical Composition	***Risicnius comminius* (Ogiri-Okpei)**
Phytate	4.36 ± 0.06
Tannin	0.64 ± 0.02
Saponin	0.34 ± 0.02
Flavonoid	0.13 ± 0.01
Alkaloid	0.84 ± 0.00
Phenol	0.08 ± 0.00
Steroid	0.08 ± 0.00
Total	**1.06 ± 1.57**

Values are means and standard deviations of three replicates

Table 4.2: Proximate composition of *Risicnius comminius* seeds

Proximate Composition	***Risicnius comminius* (Ogiri-Okpei)**
Moisture Content (%)	35.77 ± 0.10
Dry Matter (%)	64.23 ±0.10
Ash (%)	7.71 ± 0.13
Crude Fibre (%)	1.85±0.00

Ether Extract (%)	5.62 ± 0.00
Crude Protein (%)	7.66 ± 0.25
Total	**4.97 ± 2.66**

Values are means and standard deviations of three replicates

Table 4.3: Vitamin composition of *Risicnius comminius,*

Vitamins	***Risicnius comminius* (Ogiri-Okpei)**
Thiamine (B_1)	0.04 ± 0.00
Riboflavin (B_2)	0.09 ± 0.00
Niacin (B_3)	0.07 ± 0.00
Retinol (A)	6.25 ± 0.00
Tocopherol (E)	5.35 ± 0.08
Ascorbic Acid (C)	4.78 ± 0.03
Total	**2.76 ± 2.85**

Values are means and standard deviations of three replicates

Table 4.4: Mineral composition of *Risicnius comminius* seeds

Mineral Composition	***Risicnius comminius* (Ogiri-Okpei)**
Calcium	180.23 ± 0.06
Magnesium	54.36 ± 0.03
Sodium	26.38 ± 0.41
Potassium	284.62 ± 0.41
Iron	2.72 ± 0.02
Phosphorus	216.36 ± 0.04
Total	**127.45 ± 115.33**

Values are means and standard deviations of three replicates

Table 4.5: Trace metal composition of *Risicnius comminius* seeds

Trace metal	***Risicnius comminius* seeds (ppm)**
Pb(II)	**0.460**
Cr(III)	**0.056**
Zn(II)	**0.840**
Fe(II)	**1.790**
Cd(II)	**0.016**

The vitamin composition of *Ricinus communis* is shown in Table 4.3. 5.35 ± 0.08 mg/100 g of vitamin A, the relatively high concentration of vitamin A makes it good for the body. This

vitamin is responsible for improved vision. *Ricinus communis* contains 5.35 ± 0.08 mg/100g of vitamin E, this vitamin acts as antioxidants (vitamin E) which may protect against some forms of cancer. 4.78±0.03 mg/100g are mean value of vitamin C for *Ricinus communis*. Vitamin C has anti-infective properties, promotes wound healing, may boost the immune system and may help ward off infections (Wright, 2000). The mean values of the B-complex vitamins (B_1, B_2, and B_3) are 0.04 ± 0.00, 0.09 ± 0.00 and 0.07 ± 0.00 . [10] Reported 0.09±0.01, 0.22±0.01 and 0.38±0.01 respectively for the same B-vitamins. The role of these B-vitamins cannot be overemphasized. B_1 is often called an anti-stress vitamin because of its ability to protect the immune system. The rich micro-nutrient content of the spices makes them beneficial and useful to the physiological needs of man.

Table 4.4 shows the result of the minerals present in *Ricinus communis* 314.28 ± 0.11 of potassium (k) and 216.36 ± 0.04 of phosphorus present in the samples. These result is in close agreement with the findings of [10]. Table 4.5 shows trace metal composition of *Ricinus communis* seeds.

CONCLUSION

Ricinus communis seed was studied and the results shows the presence of protein, crude fibre, ash, minerals and vitamins in appreciable quantities. It revealed that the seeds are rich in phytochemicals, most amount of alkaloids, saponins, and flavonoids which has demonstrated a wide range of biochemical and pharmacological effects including anti carcinogenic, anti-therogenic, anti-inflammatory, anti-oxidant activities, suggesting and promising to have a long term health benefits. *Ricinus communis* seed have been screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. As regards the presence of anti-nutrients observed, it should be noted that most anti nutrients are destroyed or reduced to non-significant level by normal food processing methods of washing and boiling. Therefore, the consumers are not at any reasonable risks.

REFERENCES

1. Achi, O. K (2005), Traditional Fermented Protein Condiments in Nigeria, African Journal of Biotechnology. Vol. 4(13), pp. 1612-1614.
2. Odibo, F. J.C., Ezeaku, E. O and Ogbo, F.C. (2008), "Biochemical Changes During the Fermentation of *propis Africiana seeds.* For Ogiri-okpei production. Journal of Industrial Microbiology Biotechnology.
3. AOAC (1990). Official Methods of Analyses, Vitamins and Other Nutrients. 16th Edu. AOAC, Washington DC, Chapter 45, pp. 1058-1059.
4. Harborne, J. B (1997). Phytochemical Methods: A Guide to Modern Techniques in Plant Analysis. Chapman and Hall. London, Press. P. 26.
5. Okwu, D. E., Morah, F. N. (2004). Mineral and Nutritive Value of *Dennettia Tripetala* Fruits. Fruits 59 (6): 437-442.
6. Rimm, E. B., Katan, M. B. and Ascherius, A. (1999). Relation between Intake of Flavonoids and Risk for Coronary Heart Disease in Male Health Professionals: Ann Int. Med. 125: 384-389.
7. Okwu,D.E. (2006). Potential of Ocimium gratissimum, pengluna extensa and tetrapntra as spice and flavouring agents. Journal of Chemical Society of Nigeria. Vol. 31(1 & 2): 38-40.
8. Sofowora, A. (1982). Medicinal Plants and Traditional Medicine in Africa John Wiley and Sons Ltd: pp. 1-10.
9. Ashok, K and Upadhyaya K (2012), 'Tannins are astringent''. Journals of Pharmacognosy and Phytochemistry, IC Journal Issue 3, vol. 1, p. 49
10. Abii. T.A. (2009). Evaluation of the Phytochemicals, Proximate

Composition, Mineral and Vitamin Contents of Xylopia Aethopica and Monodora myristica. International Resources Journal in Engineering Science and Technology. (IREJEST) vol. 6. No.1.

11. Pal. D and Verma. P(2013), Flavonoids: A Powerful and Abundant Source of Antioxidants, International Journal of Pharmacy and Pharmaceuticals Science, vol.5, Issue 3, p. 97.

12. Sala. N. Muller, N.J., Pagange, G. Tijiburg, L., Boluese, G. P., Rice, E., and Evans and Evans, C;(1995). Polyphenolic Flavonoids as Scavenger of Aqueous Phase Radicals as Chain Breaking Antioxidant: Arch. Biochem. Brch. 2. 339-346.

13. Evans, W.C. (1989). Trease and Evans Phamacognosy 13th (ed) Balliere Tindal, Lonodon. Pp. 419-420.