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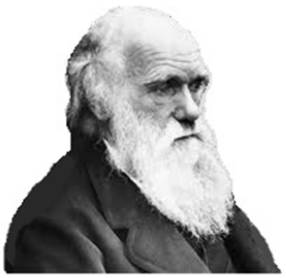

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Phytochemicals, nutraceuticals and antinutritional factors assessment of young leaves of *Colocasia esculenta* (L) Schott

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ABSTRACT

Phytochemicals, proximate, minerals and anti-nutrient compositions of young cocoyam (*Colocasia esculenta*) leaves were determined using standard methods. Qualitative and quantitative analyses for phytochemicals confirmed the presence of bioactive constituents such as alkaloids, flavonoids and saponins in appreciable amounts, while terpenes, cardiac glycocides and tannins were only present in trace concentrations. Mineral analysis revealed that K recorded the highest content - with 214.00 ± 2.11 mg/100g, followed by Ca - 157.10 ± 1.47 mg/100g, Mg - 63.00 ± 0.58 mg/100g, P - 32.00 ± 0.61 mg/100g, while Fe had the lowest value - 0.10 ± 0.14 mg/100g. Proximate composition analysis of *C. esculenta* leaves indicated that CHO, protein and crude fibre were present in high quantities (35.22, 17.10 and 16.41 % respectively), while fat was at the lowest content (8.82%). Anti-nutritional factor analysis of young leaves of *C. esculenta* indicated that phytates was present in huge quantity - 11.03 ± 0.12mg/g, followed by oxalates - 7.62 ± 0.14 mg/g, while tannins recorded the lowest anti-nutritional contents - 0.12 ± 0.06 mg/g. This study has also revealed that the young leaves of *C. esculenta* contain appreciable levels of bioactive components (phytochemicals such as as alkaloids, flavonoids and saponins; minerals K, Ca, Mg, P and Fe) and appreciable amounts of CHO, protein and crude fibre. The results of anti-nutrients analysis showed high contents of phytate and oxalate. The last is probably responsible for the itching effect and also interferes with the utilization of essential nutrients. Thus, young leaves of *C. esculenta* provide appreciable quantities of nutrients and thus can rival other conventional vegetables normally consumed in Nigeria.

***Keywords*:** Antinutrients, *Colocasia esculenta*, minerals, oxalates, phytates, phytochemicals, proximate

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1. INTRODUCTION

Medicinal plants have played a noteworthy role in various ancient traditional systems of medication. Recently, plants provide an inexpensive source of drugs for majority of world's population. The medicinal significance of a plant is due to the existence of chemical constituents like alkaloids, glycosides, resins, volatile oils, gums, tannins etc. Plant based substances have recently become of great interest owing to their resourceful applications. These compounds are synthesized by primary or rather secondary metabolism of living organisms [1]. Phytochemicals may have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories [2].

Fig. 1. *Colocasia esculenta* (L.) Schott

In Nigeria, there have been reported cases of malnutrition problems although different in magnitude and severity among different areas are due to protein, vitamins, iron, and other mineral deficiency. This situation calls for identifying a broader range of plant species which have the potentials as major food sources and for developing these for efficient food production in the Nigerian economy [3].

Colocasia esculenta (also known as "Ikpong" by the Ibibio tribe in Southern Nigeria) is a member of the *Araceae* family. It is an ancient crop grown throughout the humid tropics for its edible corns and leaves, as well as for other traditional uses. The *Aracaea* family is made up of some hundred genera and more than fifteen hundred species [4]. They are mostly tropical and subtropical. They are cultivated mainly in moist or shady habitats. Some are terrestrial while others are vines, creepers or climbers. Cocoyam is a well known food plant which has a long history of cultivation. Its corns are important source of starch. The corns can be cut up and boiled in curries or fried to make crispy chips. The leaf stalk and matured leaves are also eaten as vegetables. The Ibibios/Efiks of Southern Nigeria use the young leaves of *C. esculenta* to wrap the grated corm; this forms the very popular local delicacy known as "ekpang nkukwo".

Thus, this study evaluates the qualitative and quantitative phytochemicals, nutraceuticals and antinutrients of *C. esculenta* young leaves.

2. MATERIALS AND METHODS

2. 1. Phytate Determination

4g of the samples was taken and soaked in 100 ml of 2% HCl for 3 hours; it was then filtered through Whatman filter paper. 25 ml of the filtrate was place in 250 ml conical flask followed by the addition of 5 ml of 0.3% Ammonium thiocyanate solution as indicator. 53.5 ml of the distilled water was added to give the desired acidity. This was then titrated with standard iron (III) chloride solution which contains about 0.00195g of iron per ml until a brownish yellow persists for 5 minutes [5].

$$\% \text{ Phytic Acid} = 8.24t \times 100/1000 \times \text{wt of sample}$$

where: t = titre value

2. 2. Oxalate Determination

To about 1g of the sample was added 75 ml of 1.5N H_2SO_4 and the solution was carefully stirred using a magnetic stirrer for 1 hour before being filtered using Whatman No. II filter paper. 25 ml of the extract was collected and titrated when hot against 0.1N $KMnO_4$ solution to a faint pink colour end point [7].

$$\text{Oxalate} = (\text{titre value} \times 0.9004)\ \text{mg/g}$$

2. 3. Tannin Determination

About 0.2g of the sample was soaked in 10 ml of 70% acetone, and then placed in an ice bath to prevent the acetone from evaporating. The set up was shaken for 12 – 15 minutes to extract the Tannin. The solution was allowed to cool for about 30 minutes and then filtered to collect the supernatant. 0.5 ml of the supernatant was placed in a test tube and 0.5 ml of distilled water was added followed by the addition of 0.5 ml of Folins' reagent, 2.5 ml of 20% Na_2CO_3 solution was added, the test tube was vortexed and incubated at room temperature for 40 minutes. The resulting solution was read at 725 nm on coming calorimeter model 253;

standard Tannic acid curve was equally plotted, while the concentration of the sample was extrapolated from the plot.

2. 4. Phytochemical Screening

The experiment was carried out in the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo. The phytochemical screening involves the simple chemical test to detect the presence of secondary metabolites. The methods of Adeneye *et al.* [7], Sofowora [8] and Trease and Evans [9] were used for qualitative and quantitative phytochemical screening. The phytochemical test include: tests for saponins, tannins, flavonoids, alkaloids, cardiac glycosides and terpenes.

2. 5. Mineral Analysis

Wet Digestion of Sample: For wet digestion of sample, 2 g of the plant samples was taken in digesting glass tube. Twelve (12) ml of hydrochloric acid was added to the plant samples. The mixture was kept overnight at room temperature. Four (4.0) ml perchloric acid (PCA) was added to these mixtures and was kept in the fumes block for digestion. The temperature was increased gradually, starting from 50 °C and increasing up to 150 °C. The digestion was completed in about 70 - 85 minutes as indicated by the appearance of white fumes. The mixture was left to cool and the contents of the tubes were transferred to one hundred millilitres (100 ml) volumetric flasks and the volumes of the contents were made to one hundred millilitres (100 ml) with distilled water. The wet digested solution was transferred to plastic bottles and labelled accurately. The digest was stored and used for mineral determinations [10].

Mineral contents: calcium (Ca), magnesium (Mg), potassium (K), iron (Fe) and phosphorus (P) of plant samples were determined by atomic absorption spectrophotometer (AAS) and flame photometry according to the methods of AOAC [11] and Khan *et al*. [12].

2. 6. Determination of Proximate Content

2. 6. 1. Determination of Crude Protein

Protein in the sample was determined by Kjeldahl method. The samples were digested by heating with concentrated sulphuric acid (H_2SO_4) in the presence of digest mixture. The mixture was then made alkaline. Ammonium sulphate thus formed, released ammonia which was collected in 2% boric acid solution and titrated against standard HCl. Total protein was calculated by multiplying the amount of nitrogen with appropriate factor (6.25) and the amount of protein was calculated. Percent crude protein content of the sample was calculated by using the following formula: % Crude Protein = 6.25* × %N (*Correction factor) [13].

$$\% \text{ N} = \frac{(\text{SB}) \times \text{N} \times 0.014 \times \text{D}}{\text{W}_{\text{t.}} \text{ of the sample} \times \text{V}} \times \frac{100}{1}$$

where:
S = Sample titration reading
B = Blank titration reading
N = Normality of HCl

D = Dilution of sample after digestion
V = Volume taken for distillation
0.014 = Milli equivalent weight of Nitrogen

2. 6. 2. Determination of Ash

For the determination of ash, clean empty crucible was placed in a muffle furnace at 600 °C for 1 hour, cooled in desiccator and weight of empty crucible was noted (W_1). 1g of each sample was taken in crucible (W_2). The sample was ignited over a burner with the help of blowpipe, until it charred. The crucible was placed in muffle furnace at 550 °C for 2 – 4 hours. The appearance of grey white ash indicated complete oxidation of all organic matter in the sample. After ashing, furnace was switched off. The crucible was cooled and weighed (W_3). Percent ash was calculated by following formula:

$$\% \text{ Ash} = \frac{\text{Difference in W}_{\text{t.}} \text{ of Ash}}{\text{W}_{\text{t.}} \text{ of Sample}} \times \frac{100}{1}$$

Difference in wt. of Ash = $W_3 - W_{1.}$

2. 6. 3. Determination of Crude Fat (Lipid)

Dry extraction method for fat determination was used. It consisted of extracting dry sample with some organic solvent, since all the fat materials e.g. fats, phospholipids, sterols, fatty acids, carotenoids, pigments, chlorophyll etc. are extracted together. Therefore, the results are frequently referred to as crude fat. Fats were determined by intermittent soxhlet extraction apparatus. Crude fat was determined by ether extract method using soxhlet apparatus. Approximately one gramme (1g) of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. Weighed, cleaned and dried the receiving beaker was filled with petroleum ether and fitted into the apparatus. Turn on water and heater to start extraction. Siphoning allowed ether to evaporate and disconnect beaker before last siphoning. The extract was transferred into clean glass dish with ether washing and evaporated ether on water bath. Then the dish was placed in an oven at 105 °C for 2 hours and cooled in a desiccator.

The percentage crude fat was determined by using the following formula:

$$\% \text{ Crude fat} = \frac{\text{W}_{\text{t.}} \text{ of ether extract}}{\text{W}_{\text{t.}} \text{ of sample}} \times \frac{100}{1}$$

2. 6. 4. Determination of Carbohydrates (CHO)

The carbohydrate content was obtained by the difference (i.e. subtracting the values obtained for crude protein, crude lipid, crude fibre and ash from 100) as proposed by Adeola *et al*. [14].

2. 6. 5. Determination of Crude Fibre

A moisture free and ether extracted sample was first digested with dilute H_2SO_4 and then with dilute KOH solution. The undigested residue collected after digestion was ignited

and the loss in weight after ignition was registered as crude fibre. Calculations were done by using the formula:

$$\% \text{ Crude Fibre} = \frac{W_1 - W_2}{W_0} \times \frac{100}{1}$$

W_1 = Weight of undigested residue
W_2 = Weight after ignition
W_0 = Weight of sample

2. 6. 6. Determination of Moisture Content

An empty evaporating dish was washed and dried in the oven, allowed to cool in the desiccator and weighed (w_1). About 3g of the sample was weighed into the dish and recorded as (w_2). The sample plus evaporating dish was transferred into the oven maintained at 105 °C and kept there for 3 hours. The sample was then removed, allowed to cool in the desiccator and then weighed. This process was continued until a constant weight was obtained and recorded as:

$$\% \, Moisture \; content = \frac{(\text{weight loss due to drying}) \times 100}{(\text{weight of sample})}$$

$$\% \, Moisture \; content = \frac{(W2 - W3) \times 100}{(W2 - w1)}$$

3. RESULTS AND DISCUSSION

Results of mineral nutrients analysis of young leaves of *C. esculenta* revealed appreciable composition of mineral elements (Table 1). K recorded the highest content with 214.00 ± 2.11 mg/100g, followed by Ca (157.10 ± 1.47 mg/100g), Mg (63.00 ± 0.58 mg/100g), P (32.00 ± 0.61 mg/100g) while Fe had the lowest value with 0.10 ± 0.14 mg/100g. These results are similar to the results obtained by Olaye *et al*. [15], Marcel and Jean [16]. The significantly high potassium, calcium, magnesium and phosphorus content in the samples indicate that the cocoyam leaves are good for excellent nerve function, osmotic equilibrium and bone development [15].

Table 2 shows details of the proximate composition of *C. esculenta* leaves. CHO, protein and crude fibre were present in high quantities (35.22, 17.10 and 16.41 % respectively), while fat recorded the lowest content with 8.82% (Table 2). Similar results were observed by Odedeji *et al.* [17]. The low fibre content of the vegetable could prevent intestinal irritation; improve digestibility and overall increase in nutrient utilization [17,18].

Okon *et al*. [19] reported that a diet low in fibre is undesirable and could cause constipation; hence many diets are associated with disease of colon like piles, appendicitis and cancer. The high content of carbohydrate shows that *C. esculenta* leaves is a good source of energy [15].

Table 1. Mineral Composition of young leaves of *Colocasia esculenta.*

Mineral components	*Colocasia esculenta* (mg/100g)
Potassium (K)	214.00 ± 2.11
Calcium (Ca)	157.10 ± 1.47
Magnesium (Mg)	63.00 ± 0.58
Phosphorus (P)	32.00 ± 0.61
Iron (Fe)	0.10 ± 0.14

Table 2. Proximate Composition of young leaves of *Colocasia esculenta*

Proximate contents	*Colocasia esculenta* (%)
Carbohydrate (CHO)	35.22
Protein	17.10
Fat	8.82
Crude fibre	16.41
Ash	12.61
Moisture Content	9.85
Caloric Value (Kcal)	301.20

Figure 2 shows the Quantitative analysis of Phytochemicals (%) of the young leaves of *Colocasia esculenta*. Alkaloids, flavonoids and saponins recorded 2.31, 1.52 and 1.47% respectively, while tannins and cardiac glycocides had the lowest quantitative phytochemical contents (Figure 2).

Qualitative analysis of phytochemicals of *C. esculenta* young leaves revealed that alkaloids was present in high concentration, flavonoids and saponins were moderately present, terpenes and cardiac glycocides were present in trace quantities while tannins was absent (Table 3).

Krishnapriya and Suganthi [20], Olaye *et al*. [15] also reported similar observations. In humans and most animals, alkaloids and flavonoids have been observed to possess antidiuretic, antispasmodic, anti-microbial, anti-inflammatory and analgesic effects [21].

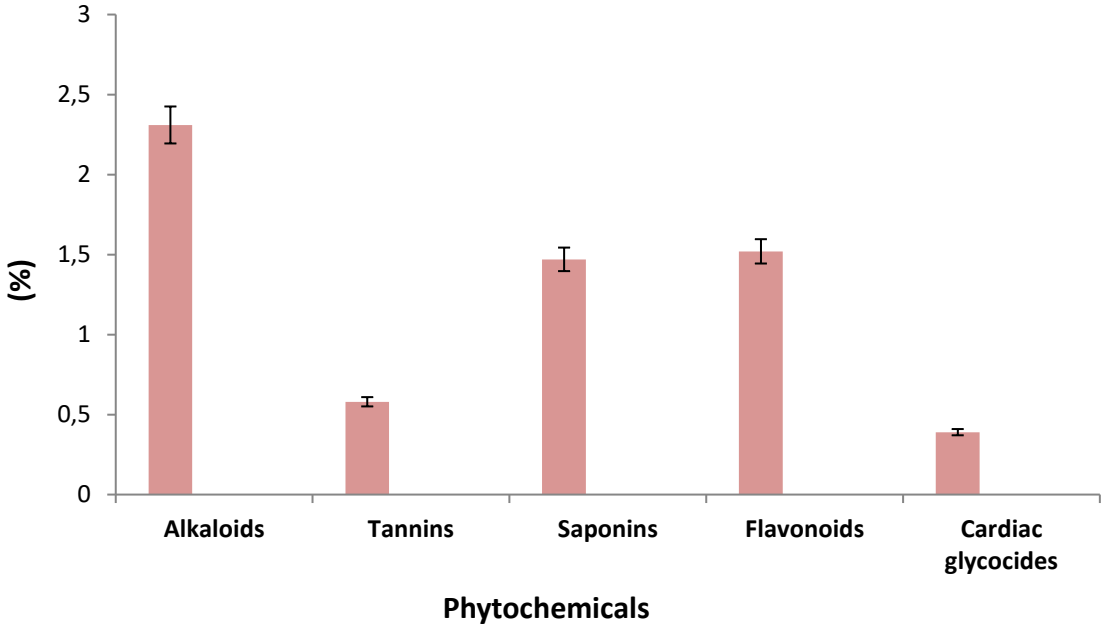

Fig. 2. Quantitative Phytochemical Composition of *Colocasia esculenta*

Table 3. Qualitative Phytochemical Contents of *Colocasia esculenta*

Parameters	Inference
Alkaloids (Dragendorff's reagent)	+++
Flavonoids (Ammonia test)	++
Saponins (Frothing test)	++
Tannins (Ferric chloride test)	-
Terpenes	+
Cardiac Glycocides (Keller-Killani Test)	+
Cardiac Glycocides (Salkowski test)	+

+ + + = High concentration ++ = Moderate concentration + = Trace concentration - = Absent

Table 4 shows the anti-nutritional factors of young leaves of *C. esculenta*. Phytates was present in huge quantity 11.03 ± 0.12 mg/g, followed by oxalates 7.62 ± 0.14 mg/g, while tannins recorded the lowest anti-nutritional contents with 0.12 ± 0.06 mg/g. Tannins are phenolic compounds that react with proteins [17]. They are astringent and adversely affect feed intake. Tannins bring about their antinutritional influence largely by precipitation or binding dietary protein and digestive enzymes to form complex, which are not readily

digestible [17, 22]. Tannins are known to inhabit the activities of some enzymes like amylase and lipase resulting from the formation of complexes with protein [17, 22].

Table 4. Summary of Antinutrients Contents of *Colocasia esculenta*

Parameters	*Colocasia esculenta* (mg/g)
Phytates	11.03 ± 0.12
Oxalates	7.62 ± 0.14
Tannins	0.12 ± 0.06

4. CONCLUSION

From this research, it has been shown that young leaves of *Colocasia esculenta* contain appreciable level of bioactive components (phytochemicals) such as alkaloids, flavonoids and saponins; minerals K, Ca, Mg, P and Fe, appreciable amount of CHO, protein and crude fibre. The results of anti-nutrients analysis showed high contents of phytate and oxalates which is probably responsible for the itching effect. Young leaves of *Colocasia esculenta* presents us with appreciable quantities of nutrients which are needed by the body and can be consumed and thus can rival other conventional vegetables normally consumed in Nigeria.

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