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Chemical Composition of Red and White Cocoyam *(Colocosiaesculenta)* Leaves

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Abstract: ***Standard methods were employed to analyze red and white cocoyam (Colocosiaesculenta) leaves for proximate, mineral and anti-nutrients compositions. Qualitative and quantitative analysis of phytochemicals were also carried out to ascertain their presence and relative abundance in the samples. These analyses showed that the samples possess high ash content, hence, appreciablemineral content. The samples were also found to have large amounts of anti-nutrients; phytate and oxalates. Qualitative and quantitative analyses for phytochemicals confirmed the presence of bioactive constituents liketannins, flavonoids, total phenols and alkaloids in various proportions.***

Keywords: *Colocosiaesculenta*, proximate, anti-nutrients, phytochemicals

1. Introduction

Cocoyam, a member of the Aracaea family is an ancient crop grown throughout the humid tropics for its edible corms, cormels and leaves, as well as other traditional uses [1]. The Aracaea family is made up of some hundred genera and more than fifteen hundred species [2].They are mostly tropical and subtropical and grow mainly in moist or shady habitats. Cocoyam is a well-known food plant which has a long history of cultivate, it corms are important source of starch and the leaf can also be eaten.

Nutritional value is the main concern when a plant part is considered as food source. However, endogenous toxic factors characteristic of plant material can also affect the content of nutrients. Therefore, the objectives of this research were to determine the nutritive value of red and white cocoyam (*Colocosiaesculenta*) leaves by carrying out the following analysis;proximate composition and mineral analysis of red and white cocoyam leaves. Quantitative determination of anti-nutrients present in the sample. Qualitative and quantitative analysis of phytochemicals present in the samples, thus, providing consumers and nutritionists with information about the leaves.

2. Literature Survey

Green leafy vegetables are widely used for food in many countries of the world; they are real source of B-Carotene, ascorbic acid, minerals and dietary fibre [3]. However, dark are nutritious than those with lighter or yellowish leaves. They are in abundance shortly after rainy season but become scarce during the dry season which cultivated types are used, these vegetables could be harvested at all stages in the process of growth or could be fed upon in fresh, process or semi processed forms, [3].

Cocoyam is a well-known food plant, which has a long history of cultivate. Its corms are important source of starch. They may also be cut up and boil in curries or fried to make crispy chips. The leaf stalks can also be eaten. The leaves which are seldomly used for food have been reported that they can be cooked [4]. All parts of the of the raw cocoyam plant contain a toxic compound calcium oxalate, which must be destroyed through cooking before eating [5].The plant is herbaceous in nature and it is capable of growing to a height of 2meters. They seldom flower and fruit in cultivation and the flowers expectedly possesses the usual aroid flower structure. The leaves of cocoyam are shape like a shield and can reach up to a meter in length; they are attached to the long, fleshy leafy stalks that are green, red or purple. Slightly off the center of each leaf blade and such a characteristic is desert, be as peltake [6].

3. Previous Work

It has been reported that leaves from Taro (Colocosiaesculenta) are rich in vitamins and minerals. They are a good source of thaimine, ribloflavin / iron, phosphorus and zinc and a very good source of vitamin B6, vitamin C, niacin, and especially in the leaves, also it is highly perishable that is it has high content of water. Leaves of new cocoyam are also used in feeding pigs because of its nutritive values [4]

Preliminary investigations have reveal that most vegetables are good source of antioxidants, but some are definitely much better than others. When it comes to vitamins, minerals, carotenoids and cruciferous phytonutrients, which are all virtually importable to good health, the most potent vegetable are dark green leafy vegetables, hence, this effort to fully investigate the nutritional, anti-nutritional and phytochemical characteristics of leaves of cocoyam.

4. Methods / Approach

The succulent part of the red and white cocoyam leaves were plucked, washed in running tap to remove sand, stone, was sun dried and was ground into fine powdered form using electric blender. The samples were placed in sample air – tight container, well labelled for identification kept in a refrigerator at 4^{o}C prior the analysis.

4.1. Proximate Analysis

This was carried out on the blended, homogenized and refrigerated leaves samples in triplicate. The determinations include; moisture content, crude fibre, ash content, crude protein, crude fat and total carbohydrates (by difference).

Table 1: Results of Proximate Analysis and Energy Value of Red and White Cocoyam Leaves

	Results	
Parameter (%)	R C L	W C L
Moisture	7.40 ± 0.05	8.15 ± 0.05
Ash	11.60 ± 0.05	11.60 ± 0.07
Crude Fibre	17.17 ± 0.10	17.41 ± 0.05
Crude Fat	6.99 ± 0.10	9.60 ± 0.07
Crude Protein	16.41 ± 0.01	15.53 ± 0.03
Total Carbohydrates	40.43 ± 0.03	37.71 ± 0.03
Energy Value (Kcal/g)	290.27	299.36

4.1.1 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 (w_3) [7].

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

$$\% \text{ Moisture Content} = \frac{(w_2 - w_3) \times 100}{(w_2 - w_1)} \quad (1)$$

4.1.2 Determination of Ash.
A crucible with its lid was washed, rinsed, dried in the oven and allow to cool in a desiccator. The weight of empty crucible plus its lid, (w_1) was measured using an analytical balance. About 3g of the sample was put into the crucible and covered with its lid. The new weight was taken and recorded as (w_2). The crucible with its content was then transferred into the muffle furnace maintained at 550°C and kept there for about 6 hours for complete ashing. The ash obtained plus crucible was allowed to cool in the desiccator and weighed (w_3) [7].

$$\% \text{ Ash Content} = \frac{(weight\ of\ ash) \times 100}{(weight\ of\ sample)}$$

$$\% \text{ Ash Content} = \frac{(w_3 - w_1) \times 100}{(w_2 - w_1)} \quad (2)$$

4.1.3 Determination of Crude Fat
About 50g of the sample was weighed (w_1) into a clean piece of cotton cloth. The sample was then wrapped securely in the cloth by tightening a thread around it. The wrapped sample was then immersed in the thimble of the Soxhlet extractor, using n-hexane and maintained at a temperature of 60°C. This set-up was left in this condition for up to 5 hours so that all the fat in the sample was extracted. The oil was concentrated from the oil-solvent mixture by removing the defatted sample from the thimble and distilling off some, but not all of the solvent from the mixture in the flask. This extract was then exposed to air overnight for complete evaporation of the residual solvent. In the morning, the flask with its content was weighed continuously until a constant weight (w_2) was obtained. The flask was then emptied, cleaned thoroughly, oven-dried at 100°C and weighed (w_3) [7].

$$\% \text{ Crude Fat Content} = \frac{(weight\ of\ extracted\ fat) \times 100}{(weight\ of\ sample)}$$

$$\% \text{ Crude Fat Content} = \frac{(w_2 - w_3) \times 100}{(w_1)} \quad (3)$$

4.1.4 Determination of Crude Protein
Crude protein determination was carried out, using the micro-kjeldahl method. About 1.0g of the sample was weighed and transferred into the micro-kjeldahl flask. One tablet of catalyst and 10ml of concentrated H_2SO_4 were added to the sample inside the flask. The flask with its content was heated on a heating mantle inside a fume cupboard for 3 hours until the black solution turned colourless. The clear solution was diluted with distilled water and made up to 100ml. This was followed by distillation. 10ml of the resulting solution from the digest was measured and transferred into a distillation apparatus. Then, 25ml of 40% NaOH was added to the digested sample solution in order to make it alkaline. The cloudy nature of the sample solution after the addition of the 40% NaOH indicates that NaOH was in excess. 25ml of 2% boric acid was pipette into a receiving conical flask; to which 2 drops of mixed indicator was added to produce a pink colour solution. The distillation was carried out with all the joints tightened, making sure that the end of the delivery tube dipping below the boric acid solution. As the distillation proceeds, the pink colour solution of the receiver turned light green, indicating the presence of NH_3 [8]. Distillation was continued until the distillate was about 50ml after which the delivery end of the condenser was rinsed with distilled water into the receiving flask. The third step was the titration stage where the received ammonia in the boric acid was titrated against standard 0.01M HCl. A colour change of this solution from light green back to pink due to the presence of the mixed indicator that was earlier introduced in the distillation stage indicated the end point [9]. The titre value was noted and recorded.The final step was to estimate the % Nitrogen in the sample and hence the Crude Protein by multiplying that value by the general factor; 6.25.

$$\% \text{ Nitrogen Content} = \frac{(Titre\ value \times M \times 0.0014 \times Df \times Cf \times 100)}{(weiht\ of\ sample)} \quad (4)$$

where;
M = Molarity of HCl = 0.01M
Df = Dilution factor = 50
Cf = Correction factor = 10
% Crude protein = % Nitrogen x 6.25
6.25 is the general factor of plant-derived food sample.

4.1.5 Total Carbohydrates Estimation.
The Total Carbohydrates content was estimated by difference. The percentage Total Carbohydrates content is equal to the sum of the percentage Moisture, Crude Protein, Ash, Crude Fibre and Crude Fat contents, subtracted from 100.

$$\% \text{ Carbohydrates} = 100 - (\%Mo + \%As + \%Cf + \%Cp) \quad (5)$$

where;
%Mo = Percentage moisture content
%As = Percentage ash content
%Cf = Percentage crude fat
%Cp = percentage crude protein

4.1.6 Energy content
This is calculated with the formula below:
Energy (Kcal/g) contents of the samples = (%Fat x 9) + (%Protein x 4) + (% Carbohydrate x 4) (6)

4.2 Minerals Analysis

Mineral analysis, which include nutritionally valuable minerals such as: Na, K, Ca, Mg, Fe, and Mn were determined. The minerals were analysis from solutions obtained by first dry-ashing the samples at 550^{o}C and dissolved the ash in 10% (vol/vol) HCl, filtered and made up to 100ml in volumetric flasks using distilled, deionised water. Sodium and potassium were determined by flame photometry (U.K, model 405),Ca, Mg, Zn, Fe and Mn were determined by the AOAC Atomic Absorption spectrometric photometer (Varian company, USA.) [7]

Table2: Result for Mineral Analysis (mg/100g)

Parameter	*Red Cocoyam Leaves*	*White Cocoyam Leaves*
Sodium	188.20	192.00
Potassium	208.10	220.00
Calcium	150.00	130.00
Magnesium	96.00	68.00
Manganese	0.05	0.07
Zinc	0.06	0.10
Iron	0.10	0.08

4.3 Anti-nutrients Determination

Analyses were carried out for the anti-nutrients in the two leaf samples and are presented below;

Table 3: Results for Antinutrient Analysis (mg/g)

Parameter	*Red Cocoyam Leaves*	*White Cocoyam Leaves*
Phytates	10.10±0.06	9.10±0.06
Oxalates	5.83±0.04	8.28±0.07
Tannins	0.02±0.01	0.05±0.02

4.3.1 Phytate Determination

About 4g of the samples was taken and soaked in 100ml of 2% HCl for 3 hours; it was then filtered through Whatman filter paper. 25ml of the filtrate was place in 250ml conical flask followed by the addition of 5ml of 0.3% Ammonium thiocyanate solution as indicator. 53.5ml 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.

% Phytic Acid = 8.24t x100/1000 x wt of sample (7)
Where t = titre value

4.3.2 Oxalate Determination

To about 1g of the sample was added 75ml 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. 25ml of the extract was collected and titrated when hot against 0.1N KMnO4 solution to a faint pink colour end point.

Oxalate = (titre value x 0.9004) mg/g (8)

4.3.3 Tannin Determination

About 0.2g of the sample was soaked in 10ml of 70% acetone, and then placed in an ice bath to prevent the acetone from evaporating. The set up was shaken for 12-15minutes to extract the Tannin. The solution was allowed to cool for about 30minutes and then filtered to collect the supernatant. 0.5ml of the supernatant was placed in a test tube and 0.5ml of distilled water was added followed by the addition of 0.5ml of Folins' reagent, 2.5ml 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 725nm on coming calorimeter model 253; standard Tannic acid curve was equally plotted, while the concentration of the sample was extrapolated from the plot.

4.4. Qualitative Analysis of Phytochemicals

Chemical tests were carried out on the aqueous extract and on the powdered samples using standard procedures to identify the constituents and the results are presented below;

Table 4.1.4: Results for Qualitative Analysis of Phytochemicals

Parameter	*Red Cocoyam Leaves*	*White Cocoyam Leaves*
Saponins	+ve	+ve
Tannins	+ve	+ve
Flavonoids	+ve	+ve
Alkaloids	+ve	+ve
Steroids	-ve	-ve
Terpenoids	-ve	-ve
Phlobatannins	-ve	-ve
Total Phenols	+ve	+ve

Key
+ve means present
-ve means absent

4.4.1. Test for Saponins

About 0.5g of the powdered sample was shaken in a test tube for about 10minutes, frothing which persist on warming is taken as evidence for the presence of saponins.

4.4.2. Test for Tannins

About 0.5g of the each of the leaf extract was stirred with 10ml distilled water. This was filtered, while ferric chloride ($FeCl_3$) reagent was added to the filtrate. A blue – black or brownish green precipitate is taken as evidence for the presence of tannins.

4.4.3 Test for Flavonoids

About 0.5g of the extract was dissolved in diluted sodium hydroxide; solution of hydrochloric acid was then added. A yellow color (solution) that turns colorless indicates the presence of flavonoids.

4.4.4 Test for Alkaloids

About 0.5g of the sample extract was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath, 1ml of the filtrate was treated with Dragendorff's reagent. Turbidity or precipitation with the reagent is taken as evidence of the presence of alkaloids.

4.4.5 Test for Steroids

2ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2mlH_2SO_4. The colour doesn't change from violet to blue or green indicating the absence of steroids.

4.4.6. Test for Terpenoids (Salkowski test)
5ml of each extract was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form layer. A non-formation of reddish brown colouration of the interface indicates the absence of terpenoids.

4.4.7 Test for Phlobatannis
The aqueous extract of the extract of each of the samples was boiled in 1% aqueous hydrochloric acid. Non-deposition of red precipitate indicates the absence of phlobatannins.

4.5. Quantitative Analysis of Phytochemicals

The samples were analysed for phytochemicals

Table 5: Quantitative Analysis of Phytochemical (%)

Parameter	*Red Cocoyam Leaves*	*White Cocoyam Leaves*
Saponins	1.44 ± 0.08	1.50 ± 0.05
Tannins	0.47 ± 0.07	0.76 ± 0.06
Alkaloids	0.99 ± 0.10	0.75 ± 0.04
Total Phenols	0.02 ± 0.01	0.27 ± 0.02

4.5.1 Determination of Total Phenols by Spectrophotometric Method
The fat free sample was boiled with 50ml of ether for the extraction of phenolic component for 15minutes, 5ml of the extract was pipette into a 50ml flask then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amylalcohol were also added. The sample was made up to the mark and left to react for 30minutes for colour development. This was measured at 505nm.

4.5.2 Alkaloid Determination Using Harbonrne Method
About 5g of the sample was weighed into a 250ml beaker and 200ml of 10ml acetic acid in ethanol was added, covered and allowed to stand for 4hours. This was filtered and the filtrate was concentrated on 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 the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

4.5.3 Saponin Determination Using Obadoniand Ochuko Method
20g of each sample were put into a conical flask and 100cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hours with continuous stirring at about 550^oC. The mixture was filtered and the residue re-extracted with another 200ml 20% aqueous ethanol. The combined extracts were reduced to 40ml over water bath at about 90^oC. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethylether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded, the purification process was repeated. 60ml of n- butanol was added, the combined n – butanol extract was washed twice with 10ml 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; the saponin content was calculated as a percentage.

4.5.4 Tannin Determination by Van- Burden and Robinson Method
500mg of the samples was weighed into a 50ml plastic bottle; 50ml of distilled water was added and shaken for 1hr in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm.

5. Results and Discussion

Table 1 presents the proximate composition of red and white cocoyam leaves. The moisture content of the red and white cocoyam leaves was analysed to be 7.40 %and 8.15%, Ash: 11.60% and 11.60 %, Fat Content: 6.99% and 9.60%, Crude fibre: 17.17% and 17.41%, Protein content: 16.41% and 15.53% Carbohydrates: 40.43% and 37.71% respectively. The values of the moisture content were in line with the values for some vegetables in Nigeria which ranged from 7.60-8.55% [10]. The fat content of the white (9.60%) was in line with result obtained by Adeyeye and Fagbohun [11] for Solanumgilo (9.0%) and of dried leaves of Melantherascadens (9.43%) reported by Omoyeni et.al [12]. Protein value is16.41% and 15.53% low compare to dried leaves of Melantherascadens with a value of 22.58%.The crude fibre content is 17.17% and 17.41%, which was low compared to the result obtained for F. asperifolia (28.68%) and F. sycomorus (31.54%). Okon et al [14] 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 (40.43% and 37.71% in Colocossiaesculenta shows) that it is a good source of energy.

Table 2 shows the results of mineral analysis (mg/100g) shows Na (188.20/ and 192.00); K (208and 220); Ca (150 and 130); Mg(96 and 68.00); Mn(0.05 and 0.07);Zn(0.06 and 0.10)and Fe(0.10 and 0.08). These values were high compare to the amount of detectable elements in Ochthocharisdicellandroides ranging from 0.01 to 0.66% reported by Marcel and Jean [14]. The significantly high sodium, potassium and calcium content in the samples indicate that the cocoyam leaves are good for excellent nerve function, osmotic equilibrium and bone development.

Table 3 shows the Antinutrient composition in mg/g, Phytates (10.10 and 9.10); Oxalates (5.83 and 8.28); Tannins (0.02 and 0.05) for the dried red and white cocoyam leaves. Ogbe et.al [15] reported phytates, oxalates and tannins (%)to be 2.57, 0.45 and 21.10 respectively for moringa leaves. Phytate is an organically bound form of phosphorus in plants. The relatively high phytates content in the sample could reduce the availability of useful minerals present in the sample because they are in known to bind with essential minerals (such as calcium, iron, magnesium and zinc) in the digestive tract, resulting in mineral deficiencies [16].

Table 4 shows the Qualitative analysis of Phytochemicals of dried red and white cocoyam leaves revealed the presence of saponins, tannins, flavonoids, total phenols, alkaloids and the absence of steroids, terpenoids and phlobatanins in both samples and Table 5., shows the Quantitative analysis of Photochemicals (%) of the dried red and white cocoyam

leaves to be, total phenols (0.02 and 0.27); alkaloids (0.99 and 0.75); saponins (1.44 and 1.50) and tannins (0.47 and 0.76) respectively. These results were low compared to the results of dried leaves of Melantherascandens, saponins (6.42%) and alkaloids (8.90%) while the tannins content were high compare to tannic acid (0.11%) [12], but low tannins content compared to moringa leaves 21.10% [15]. The relatively high levels of saponins in the samples could result in hypocholestrolaemia, anaemia and protein deficiency because it binds cholesterol making it unavailable for absorption [17], have haemolytic activity against red blood cell [18]and forms saponin-protein complex which reduces protein digestibility [19], [20].

6. Conclusion

This study showed Colocosiaesculenta has appreciable amount of ash content, crude fiber, protein and carbohydrate content and high moisture content indicating a short shelf life. The mineral contents were high due to the high ash content. The results of anti-nutrients analysis reveal high values of phytate and oxalates. The qualitative and quantitative analyses of phytochemicals show the presence of bioactive constituents like tannins, flavonoids, total phenols and alkaloids.

7. Future Scope

Further research effort should dwell on isolation and purification of some of the useful phytochemicals that were identified in these leaves. After isolation and purification of these phytochemicals from these samples, we recommend in-vitro and in-vivo experiments to substantiate claims of earlier researchers on these bioactive compounds. Extra efforts should be geared towards determining and perfecting proper food processing techniques such as boiling, blanching etc. which could reduce or eliminate the anti-nutrients that were identified to encourage the full inclusion of cocoyam leaves in the list of vegetables in recipes for traditional cuisines.

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