International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064

Effects of Seed Coat Absence on the Chemical Composition of Croton (*Crotonpenduliflorus*) Seed and its Oil

Abass MonsuruAdeyinka[1], Owolabi Bodunde Joseph[2], Isiaka Adekunle Amoo[3]

[1, 2, 3] Department of Chemistry, Federal University of Technology, P. M. B 704, Akure, Ondo State, Nigeria

Abstract: ***Two stocks; with and without seed coat, of dried Crotonpenduliflorus seeds were analyzed using standard methods for proximate and mineral compositions. Oils were also extracted from the two different stocks and their physicochemical properties were determined. Results of these analyses were compared to study the effects of absence of seed coat on the chemical composition of croton seeds. This work revealed that the sample without coat is less susceptible to microbial degradation and rich in minerals. In addition, oil from sample with coat is not fit for human consumption and more susceptible to oxidative rancidity.***

Keywords: *Crotonpenduliflorus*, seed coat, proximate, minerals

1. Introduction

Croton is an extensive flowering plant genus in the spurge family, Euphorbiaceae. Croton seeds are commonly known as Turk's Cap (Yoruba; Awororoso, Igbo; Ogwuaki or Aki Ozura) thought to originate from Malaysia. The croton plant is a tropical evergreen plant widely distributed in southern part of Nigeria. The seeds are of a dull cinnamon-brown colour and oblong outline; they are about 12 mm. in length, and resemble castor seeds in size and shape, though, they are rather more angular in transverse section, the ventral and dorsal surfaces being separated by a prominent line. The kernel is yellowish and oily, and consists of a large endosperm enclosing papery cotyledons and a small radicle. The kernel is oily in nature and has an unpleasant taste.

Croton seed oil has been credited with medicinal potency in spite of its poisonous nature. For example, croton oil is also rubbed on the skin as rubefacient and counter irritant. Internally, croton oil is used as a powerful hydragogue, cathartic, and purgative. In excessive doses, it can cause severe purging, collapse and death. Externally, the oil is used as liniment for acute rheumatism arthritis, neuralgia and diseases of the joints [1].Hence, this research effort is centered on obtaining relevant information on the chemical composition of the croton seed and its oil with a view to establish or substantiate these claims.

2. Literature Survey

Croton seeds are obtained from the croton plant, a small tree-like shrub that grows throughout China and other parts of Asia. The seed of the plant is used medicinally. The seeds are gathered in the autumn, dried in the sun, and ground into powder. Croton plants are native to tropical Asia from India to New Guinea and Java, north into Indonesia and China. The plant is wild throughout the Philippine Islands, where it is also cultivated to a limited extent; often becoming naturalized after cultivation. Croton plants are also grown in southern California and elsewhere as an ornamental and curious plant. The plants are found in regions ranging from subtropical moist to tropical very dry forest life zones. Purging croton is reported to tolerate annual precipitation of 7.0 to 42.9 dm, annual temperature of 21.0 to 27.5°C, and pH of 4 5 to 7.5. Croton plant is a dry land plant, adaptable to most tropical climates, up to 1,500 m elevations, not particular as to soil type or texture. The plant is often grown in mixed forests, and commonly planted in and about towns. The croton plant is propagated from seed, the seed is sown directly in the forest or in seedbeds and the young plant is planted in desired places. It may be cultivated as a pure crop or as an intercrop with cacao or coffee, providing some shade [2].

3. Previous work

Extensive work has been done on investigating therapeutic uses of croton seed. For example it has been reported that croton seed oil has been used for the treatment of schistosomiasis and other intestinal parasites [3]. Internally, croton oil is used as a powerful hydragogue, cathartic, and purgative. In excessive doses, it can cause severe purging, collapse and death. Externally, the oil is used as liniment for acute rheumatism arthritis, neuralgia and diseases of the joints [1].

Similarly, the Council of Scientific Research, India reports that the oil contains 3.4% toxic resin. Of the acids, 37.0% is oleic, 19.0% linoleic, 1.5% arachidic, 0.3% stearic, 0.9% palmitic, 7.5% myristic, 0.6% acetic, 0.8% formic, with traces of lauric, tiglic, valeric, and butyric, plus some unidentified matter [4].

4. Methods/Approach

Materials: Croton seeds (*Croton penduliflorus*) used for the project work were obtained from “Oja-Oba” market in Akure, Ondo State, Nigeria. The sample was identified in the Department of Crop Science and Pest Control, The Federal University of Technology, Akure, Ondo State.

Sample preparation: Dried *Croton penduliflorus* seed samples were sorted and cleaned. This stock was divided into two. The seed coat of one part was removed before milling while the other was milled with the seed coat, i.e. with coat and without coat. Both samples of the seeds were

kept in an air-tight container and kept in the refrigerator at 4°C prior laboratory analysis.

4.1 Determination of Proximate Composition

Analysis for proximate composition was carried out on both samples with and without seed coat and presented below;

Table1: Proximate Composition of (*Croton Penduliflorus*) With and Without Seed Coat

Parameter	Sample Type	MEAN ± SD (%)
Moisture content	Without coat	3.92 ± 0.04
	With coat	6.12 ± 0.44
Ash content	Without coat	3.55 ± 0.20
	With coat	3.26 ± 0.43
Crude fat	Without coat	40.50 ± 0.99
	With coat	34.01 ± 0.14
Crude fibre	Without coat	26.14 ± 1.74
	With coat	38.50 ± 1.27
Crude protein	Without coat	0.14 ± 0.001
	With coat	0.06 ± 0.01
Carbohydrate	Without coat	25.72 ± 0.57
	With coat	18.03 ± 0.54

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) [5].

$$\% \text{ Moisture Content} = \frac{(\text{weight loss due to drying}) \times 100}{(\text{weight of sample})} = \frac{(w_2 - w_3) \times 100}{(w_2 - w_1)} \quad (1)$$

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) [5].

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

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) [5].

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

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 [6]. 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 [7]. 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.

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

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 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.

% Carbohydrates = *100 - (%Mo + % As + % Cf + % Cp)* (5)

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

4.2 Mineral Analysis

10ml of HCl was added to the crucible to dissolve the ash obtained from ash determination. It was then filtered through the filter paper into a 50ml volumetric flask. The solution was made up to 50ml with distilled water and kept for analysis using BUCK Scientific Atomic Absorption/Emission Spectrophotometer, Model 210 VGP to analyze the sample. The following results were obtained from the analysis;

Table 2: Results of Mineral Composition

Element	*Type*	*Results*
Sodium, Na	Without coat	1.146 ± 0.007
	With coat	0.862 ± 0.005
Potassium, K	Without coat	31.900 ± 0.001
	With coat	37.900 ± 0.002
Calcium, Ca	Without coat	27.900 ± 0.001
	With coat	24.400 ± 0.008
Magnesium, Mg	Without coat	15.200 ± 0.000
	With coat	17.600 ± 0.000
Zinc, Zn	Without coat	0.203 ± 0.001
	With coat	0.214 ± 0.001
Copper, Cu	Without coat	0.058 ± 0.001
	With coat	0.091 ± 0.001
Iron, Fe	Without coat	0.778 ± 0.006
	With coat	1.267 ± 0.002
Lead, Pb	Without coat	0.040 ± 0.008
	With coat	0.059 ± 0.017

4.3 Physicochemical Analysis of the Oil

The physicochemical analysis carried out on the oil includes the determination of Free Fatty Acid (FFA), Acid value, Iodine value, Saponification value and Peroxide value.

Table 3: Results of the Physicochemical Properties

Parameter	*Sample Type*	*MEAN ± SD*
Acid value (mg KOH/g)	Without coat	2.42 ± 0.34
	With coat	4.10 ± 0.35
Free Fatty Acid value (as oleic acid) (mg/g)	Without coat	1.21 ± 0.17
	With coat	2.06 ± 0.18
Iodine value (mg/g)	Without coat	29.64 ± 2.93
	With coat	28.06 ± 3.79
Saponification value (mg/g)	Without coat	51.83 ± 0.59
	With coat	27.77 ± 0.77
Peroxide value (meq/kg)	Without coat	2.10 ± 0.26
	With coat	3.43 ± 0.15

4.3.1 Determination of Acid Value

About 1g of the extracted oil was weighed into 250ml conical flask. Then 25ml of 95% alcohol was added followed by1ml of phenolphthalein indicator and the solution was then titrated with 0.1M potassium hydroxide (KOH). The colour changed from colourless to pink [5].

$$Acid\ value = \frac{Titre\ value \times 56.1}{weight\ of\ sample} \quad (6)$$

4.3.2 Determination of Free Fatty Acid (FFA) Value

The titre values obtained from the acid value determination was used for the determination of free fatty acid for the sample.

1ml 0.1M NaOH = 0.282g oleic acid [7]

$$Free\ fatty\ acid\ (FFA) = \frac{Titre\ value \times 0.282}{weight\ of\ sample} \quad (7)$$

4.3.3 Determination of Iodine Value

About 1 g of the oil sample was weighed into a dry 250ml conical flask followed by 10ml of chloroform and the flask was shaken to dissolve the oil. A 20ml aliquot of Wij's solution was pipette into the flask. The flask was covered with a cotton wool moistened with KI and kept in the dark at room temperature for 30 minutes. A blank containing all the reagents except the sample was prepared. 20ml of 20% potassium hydroxide was pipette and made up to 100ml into the solution. The iodine liberated from the solution was back titrated with 0.1M sodium thiosulphate solution ($Na_2S_2O_3$) using 1ml of 2% starch indicator. The blue black colouration disappears to colourless. The blank titration was carried out at the same time [5].

$$Iodine\ value = \frac{(b-a) \times M \times 126.9}{weight\ of\ sample} \quad (8)$$

where;
b = Titre value of blank
a = Titre value of the sample
126.9 = Molecular weight of Iodine
M = Molarity of sodium thiosulphate solution

4.3.4 Determination of Peroxide Value.

This test was carried out in the dark. About 1g of the oil sample was weighed into a clean boiling tube and 20ml of solvent mixture (20ml acetic acid + 20ml carbon tetrachloride) was added and boiled for 60 seconds. The content was poured into a titration flask containing 20ml of 5% potassium iodide (KI) and titrated with 0.002M sodium thiosulphate solution, using starch indicator (a ml). Blank titration (b ml) was also carried out [5].

$$Peroxide\ value = \frac{(b-a) \times 0.002 \times 1000}{weight\ of\ sample} \quad (9)$$

where; a = titre value of sample
b = titre value of blank.

4.3.5 Determination of Saponification Value

About 1g of the oil sample was weighed into 250ml conical flask followed by 25ml of alcoholic potassium hydroxide and refluxed for 30 minutes with occasional shaking. 1ml of phenolphthalein indicator was added to the solution and titrated while hot with 0.5M HCl solution. The blank was also carried out. The colour changed from pink to colourless [5].

$$Saponification\ value = \frac{(b-a) \times 56 \times M}{weight\ of\ sample} \quad (10)$$

where b = Titre value of blank
a = Titre value of the sample
M = Molarity of HCl = 0.5M

5. Results and Discussion

The proximate composition of the croton seed (Croton penduliflorus) as presented in Table 1, showed that the ash content of the seed sample without coat is 3.55% while that of the seed sample with coat is 3.26%. The higher ash content of the sample without coat suggests that minerals are more concentrated in the seed cotyledons because the ash content of a food sample is a reflection of the mineral contents preserved in the food material [8]. Also, the crude fat content of the sample with coat is 34.01% while that of the seed sample without coat is 40.50%. The lower value of the crude fat obtained in the former is due to the presence of seed coat which is known to contain little or no fat.

Table 2 shows the mineral composition (mg/l) in the croton seed (Croton penduliflorus). The high values of calcium and magnesium observed indicates that these samples can play a vital role in the development of bone, teeth, co-factor enzymatic reaction and nerve impulse transmission. Iron is needed for blood formation in the body. Cu and Zn in trace concentrations are important for the physiological functions of living tissues and regulate many biochemical processes in the body. Potassium was the most abundant major element in both samples while iron was the most abundant trace element. Iron and zinc are among the essential elements for humans and their daily requirements for adult are 15 and 18mg respectively [2]. Thus, the sample without coat is a good source of these minerals and consumption of the seed will help to alleviate symptoms of magnesium and zinc deficiency such as weakness, cardiac arrhythmia, poor growth, impairment of sexual development and poor wound healing [9]. This suggests that the processed seed without coat could contribute to the overall daily intake of these elements.

The results of physicochemical analysis on the oil as presented on Table 3 are sufficient for confirming the non-edibility of the oil. The acid values of croton seed (Croton penduliflorus) oil;4.10mg KOH/g and2.42 mg KOH/g for seeds with and without coat respectively, are high when compared with edible oils like coconut oil (0.6mg KOH/g) and cotton seed oil (0.6 mg KOH/g) [10]. The peroxide value of the oil extract with coat is 3.43meq/kg while that of the oil extract without coat is 2.12meq/kg. From the result obtained, the oil extract with coat have a higher tendency to go rancid upon storage for a longer period of time. Hence, it shelf live is shorter compared to the oil extract without coat.

6. Conclusion

This research work has revealed that moisture content of the seed without coat is lower than the seed with coat as such; the seed without coat is less susceptible to microbial degradation, invariably having a higher shelf life than the seed with coat. When processed properly, the seed without coat is a potential source of minerals such as; Na, K, Ca, and Mg. In addition, the oil from seed with coat is not fit for human consumption due to its high acid value and it is liable to undergo oxidative rancidity as a result of its high peroxide value.

7. Future Scope

Further research efforts should focus on anti-nutrients and phytochemical composition of the defatted seed sample because it holds the potential for compounding livestock feed. Further work should also be carried out to develop processing methods for reducing or removing toxic compounds that are identified in the course of the ant-nutrients and phytochemical studies.

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Author Profile

AbassMonsuru Adeyinka has a B.Tech degree in Industrial Chemistry from the Federal University of Technology, Akure, Nigeria. He is a young, active researcher and Member of Student Chemical Society of Nigeria.

Owolabi Bodunde Joseph has a (B. Sc) in Chemistry, (M. Sc) in Synthetic Organic Chemistry and (PhD.) in Medicinal Organic Chemistry. His main research interest is in the synthesis of medicinal organic compounds, especially sulphonamides and thiazides. He has also taken much interest in synthesis of agrochemicals such as fungicides and herbicides. He has taught Organic Chemistry at the tertiary institution for twenty years. He has written and co-authored many Chemistry books. He is a member of Chemical Society of Nigeria (CSN).

IsiakaAdekunleAmoo has a B Sc. in Industrial Chemistry, M.Sc. Analytical Chemistry and PhD. Analytical Chemistry. He has acquired over 28 years of teaching and research experience in tertiary institutions. He is a member of Chemical Society of Nigeria, Institute of Public Analysts of Nigeria, Institute of Chartered Chemists of Nigeria, Science Association of Nigeria, Solar Energy of Nigeria and Nigerian Institute of Science Laboratory Technology. He is a full-time Professor in the Department of Chemistry, Federal University of Technology Akure, Nigeria. He has graduated over 33 M. Tech, 9 PhD students and he has over 83 publications in reputable journals, both locally and internationally. He is the Editor-in-Chief of the Journal of Laboratory Science, a publication of The Nigerian Institute of Science Laboratory Technology, Federal Ministry of Science and Technology, Nigeria. He is a Professorial Assessor and External Examiner to many Universities such as; University of Benin, Benin City, Nigeria, Ladoke Akintola University of Technology, Ogbomoso, Nigeria, Fountain University, Osogbo, Nigeria, University of Western Cape in South Africa, etc. He is a member of Nigerian Universities Commission Accreditation Team and also a member of Review Panel on Environmental Impact Assessment of Federal Ministry of Environment, Nigeria. He is the President, Conference of Heads of Chemistry Departments in West Africa. He is happily married with children.