

Comparative Pharmacognostic, Phytochemical, and Antioxidant Profiling of Pink and White *Cola acuminata* Seed Varieties

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ABSTRACT

Background: *Cola acuminata* is an economically and culturally important West African plant. Pink and White seed varieties are frequently used interchangeably despite limited comparative pharmacognostic and compositional evidence. This study provides a pharmacognostic evaluation of both seed varieties, examining their anatomical characteristics, physico-chemical properties, and antioxidant activities.

Methods: Authenticated Pink and White *C. acuminata* seeds were examined macroscopically and microscopically. Extractive values, proximate composition, and elemental composition were determined. The *n*-hexane extracts were profiled by GC-MS. Ethyl-acetate and methanol extracts were evaluated using DPPH radical-scavenging and ferric reducing antioxidant power (FRAP) assays.

Results: The varieties differed in colour and selected descriptive microscopic features. Methanol and water gave the highest extractive yields in the Pink variety (32%) and White variety (26%). Proximate composition did not differ significantly between varieties (all $p > 0.05$). Caffeine had a higher relative GC-MS peak area in the Pink variety (68.27%) than in the White variety (56.77%). The White methanol extract showed the lowest DPPH IC₅₀ value (21.28 µg/mL), whereas the Pink methanol extract showed the highest observed FRAP value (187.45 µM FeSO₄/g dry sample).

Conclusion: Pink and White *C. acuminata* seeds showed comparable proximate composition but descriptive differences in pharmacognostic features, extractive yield, chemical profile, and *in vitro* antioxidant responses. Further replicated chemical analyses are required to confirm these observations.

Introduction

Cola acuminata is a tropical West African species whose seeds are commonly known as kola nuts. The seeds have cultural, economic, and traditional uses and are widely consumed for their stimulant properties, which are associated principally with methylxanthine constituents, including caffeine¹⁻³. Kola seeds are also used in beverages and traditional preparations, and their commercial importance has stimulated continued interest in their

composition, quality, and standardisation^{2,4,5}.

Pink, White, and Red colour forms of *C. acuminata* are recognised in Nigerian kola commerce^{5,6}. Although these forms may occur within the same species, seed colour may influence selection for cultural, commercial, or traditional use. Previous studies have reported variation in the physical, proximate, chemical, and mineral characteristics of Nigerian *C. acuminata* and *C. nitida* nuts⁵. Biochemical and phytochemical differences among *C. acuminata*

varieties have also been described⁶, while pharmacognostic and phytochemical studies have identified microscopic characters and bioactive constituents in *C. acuminata* extracts^{7,8}. Genetic variation within *Cola* species has further been reported using molecular markers¹⁹.

Despite these reports, integrated comparative information on the pharmacognostic features, extractive values, proximate composition, elemental profile, *n*-hexane GC-MS profile, and *in vitro* antioxidant responses of Pink and White *C. acuminata* seed varieties remains limited. The present study therefore compared these varieties using complementary pharmacognostic, physicochemical, chemical, and antioxidant approaches. The study is intended to provide descriptive information useful for authentication and future chemical investigation; it does not evaluate clinical efficacy, safety, or therapeutic benefit.

Materials and Methods

Collection, identification, and preparation of plant material:

Pink and White *C. acuminata* seeds were purchased from *Ipata* Market, Ilorin, Kwara State, Nigeria. The samples were authenticated at the Herbarium Unit, Department of Plant Biology, University of Ilorin, Nigeria, and a voucher specimen was deposited under number UILH/001/701/2022. The seeds were sorted according to variety, air-dried at room temperature to constant weight, pulverised separately into fine powder, and stored in sealed polythene bags until analysis.

Macroscopic and organoleptic evaluation: Fresh mature seeds of both varieties were evaluated for colour, texture, shape, odour, taste, length, breadth, and thickness using standard pharmacognostic procedures⁹. The organoleptic characters were recorded descriptively.

Microscopic evaluation: Free-hand transverse sections of Pink and White seed samples were prepared, counterstained with Safranin O, mounted on glass slides, and examined with a compound microscope. Photomicrographs were obtained at 10×, 40×, 100×, and 400× magnification. Microscopic observations were interpreted cautiously with reference to standard botanical microscopy and seed-anatomy sources^{10 - 12}. No histochemical test for lignin was performed.

Extract preparation and extractive-value determination: Five grams of powdered material from each variety were macerated separately in 100 mL of *n*-hexane, ethyl acetate, methanol, or distilled water. The mixtures were intermittently shaken for 72 h, filtered, and

concentrated under reduced pressure to constant weight. Extractive-value determination was performed in three replicate. Percentage yield was calculated as:

$$\% \text{ Yield of extract} = \frac{\text{Weight of extract}}{\text{Weight of plant material}} \times 100\%$$

DPPH radical-scavenging assay: DPPH radical-scavenging activity was determined according to Baliyan et al.¹³. A DPPH solution (80 µg/mL) was prepared by dissolving 8 mg of DPPH in 100 mL of methanol. Ethyl acetate and methanol extracts were evaluated at 10, 20, 50, 100, and 150 µg/mL. Equal volumes (100 µL) of DPPH solution and extract were mixed in a 96-well microplate and incubated in the dark at room temperature for 30 min. Absorbance was measured at 514 nm. The control consisted of DPPH solution mixed with methanol.

$$\text{Inhibition (\%)} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

IC₅₀ values were obtained from concentration-response curves. Aqueous extracts were not evaluated by the DPPH assay.

Ferric reducing antioxidant power assay: Ferric reducing antioxidant power was determined using the FRAP procedure described by Benzie and Strain¹⁴. Fresh working reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), 2,4,6-tripyridyl-*s*-triazine (TPTZ; 10 mM in 40 mM HCl), and ferric chloride (20 mM in water) in a 10:1:1 ratio. Ethyl acetate and methanol extracts were tested at 10, 20, 50, 100, and 150 µg/mL. Twenty microlitres of extract were mixed with 280 µL of FRAP reagent in a 96-well plate, incubated at 37 °C for 30 min, and read at 593 nm. Values were calculated from a ferrous sulphate calibration curve and expressed as µM FeSO₄/g dry sample. Aqueous extracts were not evaluated by the FRAP assay.

Proximate Analysis: The proximate composition of Pink and White *Cola acuminata* seed samples was determined using standard AOAC International¹⁵ procedures and relevant AOCS methods. Moisture and ash contents were determined gravimetrically. Crude fibre was measured by the standard digestion method, while total nitrogen was determined by the micro-Kjeldahl method. Crude protein was calculated as total nitrogen × 6.25. Carbohydrate content was estimated by difference: 100 – (% moisture + % ash + % crude lipid + % crude protein + % crude fibre). All results were expressed as percentages.

Elemental Analysis: Elemental analysis of the White and Pink varieties of the nuts of *C. acuminata* was performed using the standard procedure described by Nagori et al., 2025¹⁶. The trace elements found in the sample solutions

were identified using an atomic absorption spectrometer (Model 230ATS) provided by Light Path Optical Ltd, United Kingdom. In short, the sample was treated to nitric acid digestion before being submitted to an atomic absorption spectrophotometer (Model: BUCK Scientific ACCUSYS211 AAS) with a cathode lamp that was changed to identify various metals. The unique absorption wavelength of each element was determined and recorded for the two varieties.

Gas Chromatography-Mass Spectrometry (GC-MS)

Analysis: The phytochemical investigation of the *n*-hexane extract of *Cola acuminata* was performed using GC-MS (Thermo Scientific Co.) equipment. The parameters of the GC-MS instrument are as follows: Injection source (PAL Sampler), injection volume (2 μ L), column (Agilent technologies-HP-5MS), column dimension (30 m x 250 μ m x 0.25 μ m), initial temperature (50°C), program temperature (260 °C), initial pressure (9.05 psi), average velocity (38.724 cm/sec), holdup time (1.2912 min), run time (82.286 min). The sample dissolved in HPLC-grade *n*-hexane was run in full and compared using Database-Spectrum MS-NW-1798. Suggestions with the highest library match score greater than 80 % were selected, and the compounds were identified based on a comparison of their mass spectra data to those generated under identical experimental conditions by applying a two-dimensional search algorithm, considering the mass spectral similarity, as well as the retention index¹⁷.

Statistical analysis: Extractive-value determination, proximate analysis, DPPH activity, and FRAP activity were performed in triplicate and are expressed as mean $\pm$ standard error of the mean (SEM). Pink and White proximate-composition values were compared for each parameter using a two-sided Welch's independent-samples *t*-test, without assuming equal variances. Statistical significance was accepted at $p < 0.05$.

The recorded SEM values for extractive yield were 0.0 after rounding; these results are therefore reported descriptively. DPPH and FRAP results are displayed as mean $\pm$ SEM with error bars. Elemental and GC-MS results are reported descriptively because they were obtained from single or non-replicated determinations.

Results

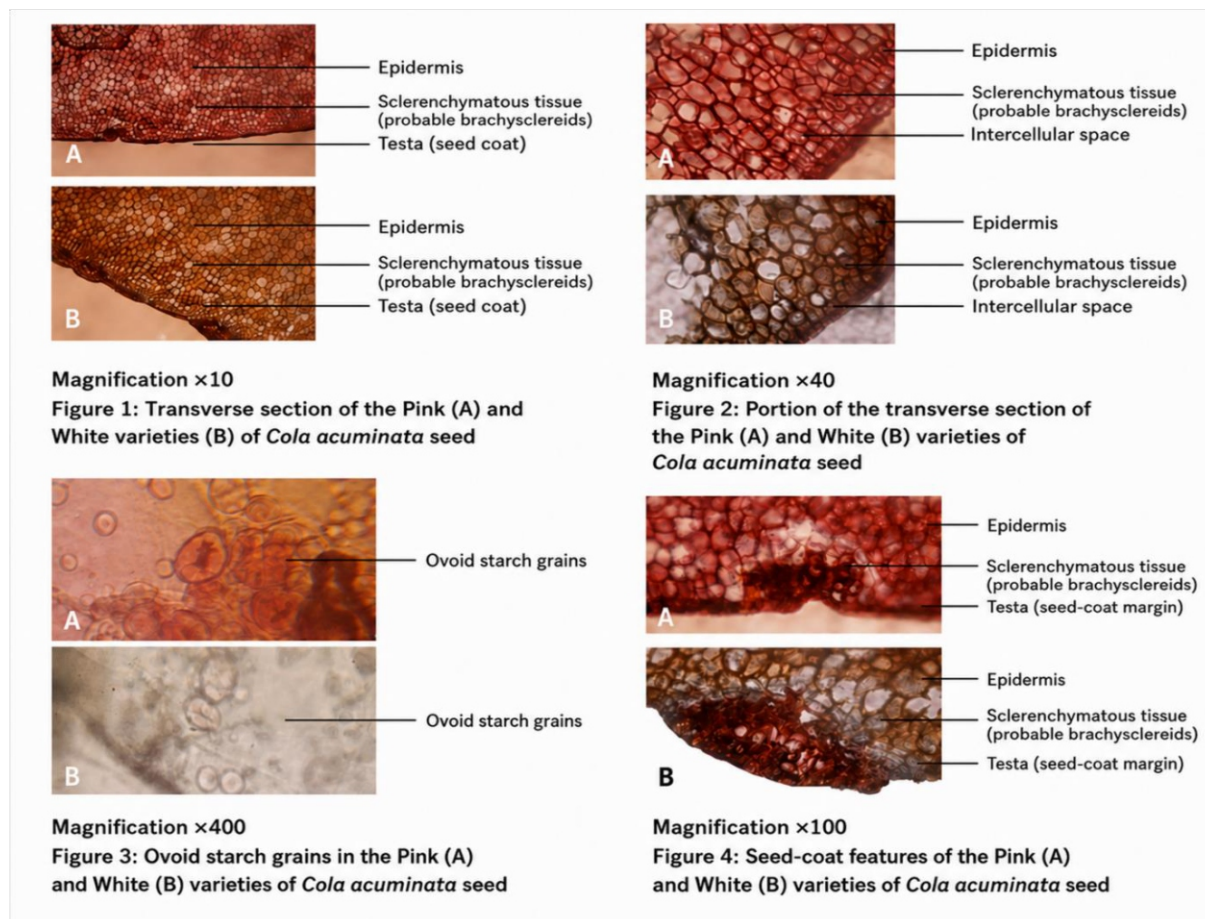
Macroscopic characteristics of *Cola acuminata* seeds:

The macroscopic characteristics of the Pink and White varieties are presented in Table 1. The Pink seeds were reddish to purplish Pink, whereas the White seeds ranged from white to brown. Both varieties had a rough, mottled surface, oval shape, nut-like odour, and a slightly bitter taste with a sweet aftertaste. Both varieties turned brown after drying. The mean dimensional measurements were similar between varieties.

Table 1: Macroscopic characteristics of Pink and White *Cola acuminata* seeds

Parameters	Pink Variety	White Variety
Colour	Reddish to Purplish pink	White to Brown
Texture	Rough, Mottled	Rough, Mottled
Shape	Oval	Oval
Odour	Nut-like aroma	Nut-like aroma
Drying Effect	Turns brown	Turns brown
Taste	Slightly bitter with sweet aftertaste	Slightly bitter with sweet aftertaste
Mean length	3.0 cm	3.1 cm
Mean Breadth	2.8 cm	2.7 cm
Thickness	1.7 cm	1.6 cm

Microscopic Description of *Cola acuminata* seeds: Transverse sections of the Pink and White seed varieties were examined at 10×, 40×, 100×, and 400× magnification (Figures 1–4). Both varieties showed an epidermis, testa, and underlying sclerenchymatous tissue interpreted as probable brachysclereids. Ovoid starch grains were observed at higher magnification. The Pink sections appeared more deeply pigmented, whereas the White sections appeared lighter. These observations are descriptive and no statistical analysis was applicable.



Extractive values

The percentage extractive yields of Pink and White seed samples are shown in Table 2. In the Pink variety, methanol and water each produced the highest yield (32.0%), followed by *n*-hexane (4.0%) and ethyl acetate (2.0%). In the White variety, methanol and water each produced the highest yield (26.0%), followed by ethyl acetate (6.0%) and *n*-hexane (2.0%). The yields were obtained from triplicate determinations. Since the reported SEM values were 0.0 after rounding, the results are presented descriptively and no inferential comparison is made.

Table 2: Extractive value of *Cola acuminata* seeds in different solvents

Solvent SAMPLE	<i>n</i> -Hexane		Ethyl acetate		Methanol		Water	
	PV	WV	PV	WV	PV	WV	PV	WV
Percentage Yield (%)	4.0±0.0	2±0.0	2±0.0	6±0.0	32±0.0	26±0.0	32±0.0	26±0.0

Values are expressed as mean ± SEM of three independent extraction determinations (*n* = 3). PV, Pink variety; WV, White variety.

DPPH Radical Scavenging Activity

Radical-scavenging activity of the ethyl-acetate and methanol extracts of Pink and White *Cola acuminata* seed varieties was evaluated by determining their IC₅₀ values. Lower IC₅₀ values indicate greater DPPH radical-scavenging activity. The ethyl-acetate extract of the Pink variety had an IC₅₀ value of 21.30 µg/mL whereas that of the white variety was 70.58 µg/mL. For the methanol extracts, the white variety recorded an IC₅₀ value of 21.28 µg/mL and the Pink variety recorded 27.66 µg/mL. Ascorbic acid, used as the reference standard, had an IC₅₀ value of 20.70 µg/mL. Among the tested seed extracts, the methanol extract of the white variety showed the lowest IC₅₀ value and was closest to the reference standard (Figure 5).

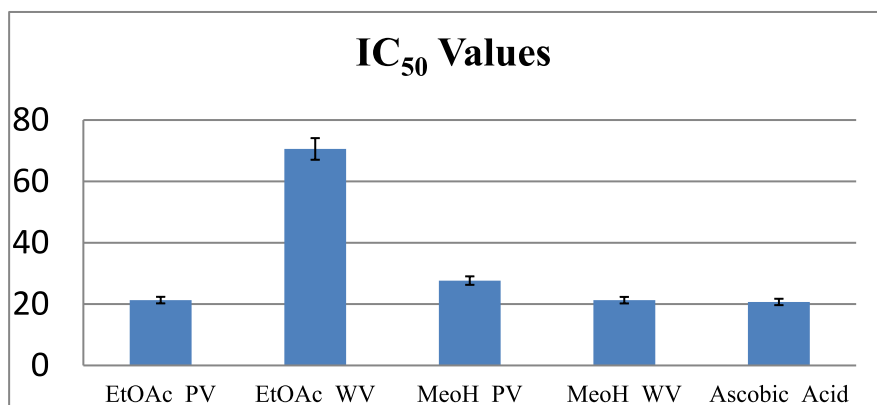


Figure 5: DPPH radical-scavenging activity of ethyl-acetate and methanol extracts of Pink and White *Cola acuminata* seeds, expressed as IC₅₀ (µg/mL). Values are expressed as mean ± SEM (n = 3). EtOAc, ethyl acetate; MeOH, methanol; PV, Pink variety; WV, White variety; AA, ascorbic acid.

Ferric reducing antioxidant power

The FRAP results are shown in Figure 6. The Pink methanol extract ranged from 31.47 to 187.45 µM FeSO₄/g dry sample across the tested concentrations. The White methanol extract ranged from 19.69 to 166.86 µM FeSO₄/g dry sample. The Pink and White ethyl-acetate extracts ranged from 12.90 to 55.75 µM FeSO₄/g dry sample and 14.57 to 59.51 µM FeSO₄/g dry sample, respectively. Ascorbic acid ranged from 9.57 to 33.78 µM FeSO₄/g dry sample. The highest observed FRAP value was recorded for the Pink methanol extract at 150 µg/mL. Error bars represent mean ± SEM from triplicate determinations; the findings are interpreted descriptively.

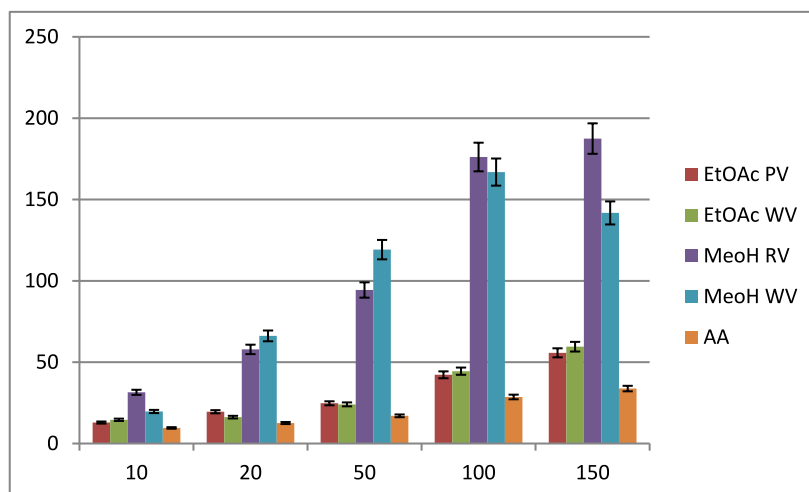


Figure 6: Ferric reducing antioxidant power (FRAP) of ethyl-acetate and methanol extracts of Pink and White *Cola acuminata* seeds at 10–150 µg/mL.

Values are expressed as mean ± SEM (n = 3). EtOAc, ethyl acetate; MeOH, methanol; PV, Pink variety; WV, White variety; AA, ascorbic acid.

Proximate composition of *Cola acuminata* seeds

The proximate compositions of the Pink and White varieties are shown in Table 3. Moisture content was $5.80 \pm 0.83\%$ in the Pink variety and $5.21 \pm 0.27\%$ in the White variety ($p = 0.558$). Carbohydrate content was $52.50 \pm 1.29\%$ and $52.34 \pm 2.02\%$, respectively ($p = 0.951$). Statistical comparison showed no significant difference between the varieties for moisture content, carbohydrate, crude lipid, crude protein, crude fibre, or total ash (all $p > 0.05$).

Table 3: Proximate Analysis of *Cola acuminata* seeds

Chemical composition	Composition (%) of Pink <i>Cola acuminata</i>	Composition (%) of White <i>Cola acuminata</i>	p-value
Moisture Content	5.8 ± 0.83	5.21 ± 0.27	0.558
Carbohydrates	52.5 ± 1.29	52.34 ± 2.02	0.951
Crude Lipid	12.8 ± 0.32	12.97 ± 0.36	0.742
Crude Protein	8.65 ± 0.46	8.86 ± 0.34	0.734
Crude Fibre	13.26 ± 1.03	13.08 ± 0.98	0.905
Total Ash	4.17 ± 0.64	4.09 ± 0.27	0.916

Values are expressed as mean $\pm$ SEM ($n = 3$). Pink and White varieties were compared using a two-sided Welch's independent-samples *t*-test. No significant difference was observed for any parameter ($p > 0.05$).

Elemental composition

The elemental composition is presented in Table 4. These values represent single determinations for each variety and are reported descriptively. The White variety had higher recorded total nitrogen, total organic carbon, potassium, sodium, manganese, iron, copper, and nickel values. The Pink variety had higher recorded phosphorus, calcium, and zinc values. Values reported as 0 mg/kg indicate that the element was not detected under the analytical conditions used.

Table 4. Elemental composition of Pink and White *Cola acuminata* seeds

Parameters	<i>Cola acuminata</i> (Pink Variety)	<i>Cola acuminata</i> (White Variety)
% Total Nitrogen	6.11	7.9
% Total Organic Carbon	55.52	71.83
Phosphorus (mg/kg)	112.3	102.6
Calcium (mg/kg)	4.67	4.1
Magnesium (mg/kg)	16.02	16.12
Potassium (mg/kg)	23.91	27.17
Sodium (mg/kg)	8.52	8.94
Manganese (mg/kg)	8.23	12.02
Iron (mg/kg)	8.75	9.46
Copper (mg/kg)	3.36	3.99
Zinc (mg/kg)	12.37	11.93
Lead (mg/kg)	0	0
Cobalt (mg/kg)	0	0
Chromium (mg/kg)	0	0
Cadmium (mg/kg)	0	0
Nickel (mg/kg)	0	14.75

GC-MS profile of the *n*-hexane extracts

Tables 5 and 6 present the putative constituents detected in the *n*-hexane extracts of the White and Pink varieties, respectively. Caffeine had the largest relative peak area in both extracts, accounting for 56.77% in the White variety and 68.27% in the Pink variety. Other detected constituents included fatty acids, fatty-acid esters, sterols, squalene, and related compounds. These findings describe the chromatographic profiles obtained under the analytical conditions used. Because the GC-MS analysis was non-replicated and compound identities were based on library matching, the results should be interpreted as tentative chemical identifications and not as quantitative confirmation of varietal differences or evidence of pharmacological activity.

Table 5: Chemical composition of the hexane extract of the white variety of *Cola acuminata* seeds

S/N	Compounds	Retention time	Relative peak area (%)
1	Octanoic acid	8.995	0.97
2	Nonanoic acid	10.338	8.93
3	8-Nonynoic acid	10.45	1.27
4	Ethyl5,8,11,14,17-eicosapentaenoate	14.604	0.34
5	Lanceol, cis	14.733	0.08
6	Ethyl5,8,11,14,17-eicosapentaenoate	14.861	0.24
7	Caffeine	15.815	56.77
8	10-Iodo-2-oxa-7-thia-tricyclo [4.3.1.0(3,8)] deca-7-oxide	16.061	0.32
9	Hexadecanoic acid, methyl ester	16.461	1.86
10	Dibutyl phthalate	16.602	0.07
11	Bicyclo [5.3.0] decane,2-methylene-5-(1-methyl vinyl)-8-methyl-7-isopropyl-1-methyl-4-methylenedecahydroazulene	16.723	0.23
12	n-Hexadecanoic acid	16.903	6.49
13	Hexadecanoic acid, ethyl ester	17.02	1.36
14	13-Tetradecynoic acid, methyl ester	17.286	0.13
15	Bicyclo[10.1.0]tridec-1-ene	17.643	1.43
16	10,13-Octadecadienoic acid, methyl ester	17.842	1.00
17	9-Octadecenoic acid, methyl ester, (E)-	18.916	1.23
18	Heptadecanoic acid, 16-methyl-, methyl ester	18.163	0.54
19	Bicyclo [10.1.0]tridec-1-ene	18.24	0.68
20	Oleic Acid	18.357	1.16
21	Ethyl9,12-hexadecadienoate	18.464	0.41
22	17-Octadecynoic acid	18.556	1.40
23	Naphthalene, decahydro-1,4a-dimethyl-7-(1-methyl-7-(1-methyl)-[1S(1.alpha,4a.alpha,7.alpha,8a.beta.)]-4.alpha.Heudesmane	19.218	0.31
24	2-methyltetracosane	20.052	0.32
25	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	20.668	0.27
26	Octacosane	21.002	0.34
27	Hexanoic acid, heptadecyl ester	21.71	4.88
28	Eicosane	21.889	0.9
29	Hexadecanoic acid, tetradecyl ester	22.392	0.38
30	Hexadecanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester	22.562	0.21
31	Eicosane	22.714	0.48
32	9-Octadecenoic acid(Z)-,2-hydroxy-1-(hydroxymethyl) ethyl ester	23.169	1.71
33	Hexanoic acid, undecyl ester	23.375	1.19
34	Octacosane	23.49	0.45
35	Pentatriacontane	24.302	0.48
36	Squalene	24.433	1.18

Table 6: Chemical composition of the hexane extract of the Pink variety of *Cola acuminata* seeds

S/N	Compounds	Retention time	Relative peak area (%)
1	Nonanoic acid	10.207	1.61
2	Caffeine	15.763	68.27
3	Hexadecanoic acid, methyl ester	16.459	1.49
4	n-Hexadecanoic acid	16.834	2.8
5	Hexadecanoic acid, ethyl ester	17.017	0.97
6	Bicyclo[10.1.0]tridec-1-ene	17.642	3.23
7	10,13-Octadecadienoic acid, methyl ester	17.84	0.72
8	9-Octadecenoic acid, methyl ester, (E)	17.912	1.31
9	17-Octadecynoic acid	18.074	1.27
10	Bicyclo[10.1.0]tridec-1-ene	18.239	2.58
11	cis-Vaccenic acid	18.344	1.37
12	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	18.553	1.74
13	Methyl 2-octyl cyclopropene-1-octanoate	19.236	0.61
14	Floxuridine	21.704	1.5
15	Diisooctyl phthalate	22.002	0.27
16	Vitamin E	22.154	2.71
17	9,19-Cyclolanostan-3-ol, 24-methylene-, (3. Beta,23E)	23.592	0.43
18	Campesterol	23.717	0.56
19	7-Hexadecenal, (Z)	23.876	0.99
20	Stigmasterol	24.292	3.81
21	Squalene	24.427	1.78

Discussion

This study compared the macroscopic, microscopic, physicochemical, chemical, and *in vitro* antioxidant characteristics of Pink and White *Cola acuminata* seeds. The findings provide descriptive pharmacognostic and compositional information that distinguishes the varieties in selected features, while statistical analysis showed that their proximate compositions were comparable.

The reddish-to-purplish Pink variety and the white-to-brown White variety differed visibly in seed colour, providing useful macroscopic characters for identification. Pigmentation differences in Nigerian kola nuts have previously been associated with variation in polyphenol content and polyphenol-oxidase activity; however, seed colour alone cannot establish the presence or concentration of specific phytochemicals¹⁸. The rough, mottled surface, nut-like odour, and slightly bitter taste followed by a sweet aftertaste are consistent with descriptions of kola seeds in

the literature^{2,5}. Although small numerical differences were recorded in seed length, breadth, and thickness, both varieties had similar overall shape and texture. These macroscopic features may assist routine identification and quality control of *C. acuminata* seeds.

The transverse sections provided further descriptive characters for seed identification. Both varieties showed an epidermis, testa, and underlying sclerenchymatous tissue interpreted as probable brachysclereids, while ovoid starch grains were observed at higher magnification. Published pharmacognostic work on *C. acuminata* has reported starch grains, sclerenchymatous fragments, fibres, and other microscopic diagnostic elements²⁰. Standard plant-anatomy references also identify the epidermis and parenchymatous seed-coat tissues in *C. acuminata*¹¹. However, the tissue interpretation in the present study remains cautious because confirmatory histochemical staining for lignin was not performed. Microscopy cannot establish differences in

phenolic content, antioxidant activity, or other bioactive constituents; direct quantitative analysis is required for such conclusions¹². The microscopic observations should therefore be regarded as descriptive diagnostic characters rather than statistically established anatomical differences between Pink and White seeds.

Solvent selection influenced the mass of extractable material recovered from the seeds. Methanol and water produced the highest yields in both varieties, whereas *n*-hexane and ethyl acetate produced lower yields. This pattern is compatible with the extraction of relatively polar constituents by methanol and water^{8,21}. Nevertheless, equal methanol and water yields in the Pink variety do not indicate identical phytochemical composition. Extractive yield represents the total mass of soluble material recovered and cannot independently establish the concentration of phenolics, alkaloids, flavonoids, or other individual compound classes. Since the triplicate extraction values had SEM values of 0.0 after rounding, the extractive values are interpreted descriptively and no statistical significance is claimed.

The proximate analysis showed that carbohydrate was the predominant component in both seed varieties, followed by crude fibre and crude lipid. This pattern is consistent with published nutritional reports on *Cola* seeds^{21–23}. Although the White variety showed slightly higher numerical values for crude lipid and crude protein, no statistically significant differences were observed between Pink and White varieties for moisture content, carbohydrate, crude lipid, crude protein, crude fibre, or total ash (all $p > 0.05$). The two varieties therefore had comparable proximate nutritional composition under the conditions of this study.

The present results may be compared with the Nigerian kola-nut quality study of Atanda et al.⁵, which evaluated dried mature red *C. acuminata* and *C. nitida* samples obtained from major Nigerian markets. Atanda et al. reported 8.27% moisture, 13.38% crude protein, 10.80% fat, 9.68% crude fibre, and 4.00% ash for *C. acuminata*. The Pink and White samples in the present study had lower moisture and crude-protein values, higher crude-lipid and crude-fibre values, and similar ash values. These numerical differences should not be interpreted as contradictory because the studies differed in sample type, colour form, source, handling, drying history, and analytical context. Importantly, the present study assessed within-species Pink-versus-White variation, whereas Atanda et al. compared *C. acuminata* with *C. nitida*. The absence of significant differences in the present proximate data indicates that visible seed-colour variation was not accompanied by statistically detectable differences in the major nutritional constituents.

Atanda et al. also reported that *C. acuminata* had higher carbohydrate and crude-fibre contents than *C. nitida*, while *C. nitida* had higher moisture, ash, lipid, and protein values⁵. Abiodun et al. similarly reported biochemical and phytochemical variation among *C. acuminata* varieties⁶. Together, these studies indicate that kola composition may vary across species, varieties, source materials, and processing conditions. The present work extends this evidence by providing comparative pharmacognostic, extractive, GC–MS, and in-vitro antioxidant observations for Pink and White *C. acuminata* seeds.

The elemental data were generated from single determinations and are therefore descriptive. Numerical differences between Pink and White seeds cannot be interpreted as statistically significant varietal differences. The recorded nitrogen and organic-carbon values may be related to the overall nutritional composition of the seeds. Published studies have reported mineral variation among *Cola* materials^{5,6,15}. Values reported as zero for lead, cobalt, chromium, and cadmium should be described as not detected under the analytical conditions used, rather than as definitive evidence of absence or safety. The nickel value recorded for the White variety also requires confirmation through replicated analysis with defined analytical detection limits.

GC–MS of the *n*-hexane extracts detected caffeine as the compound with the highest relative peak area in both varieties, with a higher relative peak area in the Pink variety. This observation is consistent with the recognised occurrence of caffeine as a major constituent of kola^{1,3,7}. Atanda et al. reported 1.01% caffeine in whole *C. acuminata* seed material using chemical quantification⁵. That value should not be compared numerically with the present GC–MS relative peak areas because the analytical methods, sample preparation, and reporting units differ. Both studies nevertheless support the presence of caffeine as an important constituent of *C. acuminata*.

Other compounds detected in the *n*-hexane extracts included *n*-hexadecanoic acid, stigmasterol, vitamin E, and squalene. Previous studies have reported anti-inflammatory activity for *n*-hexadecanoic acid and described pharmacological properties of stigmasterol^{24, 25}. However, the present GC–MS data are qualitative or semi-quantitative and were not generated from replicated quantitative measurements. The detected compounds should therefore be interpreted as constituents of the extracts under the analytical conditions used and not as proof of pharmacological activity or statistically confirmed chemical differences between the Pink and White varieties. The antioxidant results varied according to the assay and extract evaluated. In the DPPH assay, the White methanol

extract showed the lowest observed IC₅₀ value among the seed extracts, indicating the strongest observed radical-scavenging response in that assay. In contrast, the FRAP assay showed the highest observed reducing value for the Pink methanol extract at 150 µg/mL. The DPPH and FRAP assays assess different in-vitro antioxidant mechanisms^{13,14}; therefore, the extracts were not expected to show identical ranking in both assays. Although error bars show SEM from triplicate determinations, the complete replicate-level numerical data were unavailable for formal between-group re-analysis. The antioxidant findings are consequently interpreted as comparative trends rather than statistically confirmed differences. Quantitative analysis of phenolics, flavonoids, and other antioxidant-related constituents is required to explain these observed responses.

Overall, the study provides comparative pharmacognostic, compositional, and *in vitro* antioxidant information on Pink and White *C. acuminata* seeds. The statistically supported finding is that the varieties possess comparable proximate composition. Descriptive differences in macroscopic appearance, extractive yield, GC-MS profile, and antioxidant response should be confirmed by future replicated chemical and biological studies.

Conclusion

Pink and White *Cola acuminata* seeds showed comparable proximate composition, while microscopy, extractive yield, chemical profiles, and in-vitro antioxidant responses differed descriptively. These findings support pharmacognostic characterization and replicated chemical studies but do not establish efficacy, safety, or therapeutic benefit.

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