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Chemical Composition and Antimicrobial Analysis of the Pods and Seeds of *Cola rostada* and *Cola nitida*

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ABSTRACT: The chemical composition of *Cola nitida* and *Cola rostada* pods and seeds showed average moisture content of 81.75% whereas the seeds had an average of 54.60%. Crude protein ranged from 9.60-11.91% for the pods and 10.50-11-91% for the seeds. Lipid for both was less than 2%. Fibre of the pod was 15% while that of the seed ranged from 2% to 3.6%. The toxicant levels were generally low. The plants parts had low concentration of heavy metals such as cobalt, cadmium, and lead. The phytochemical components included alkaloids, saponins, tannins, polyphenols, and reducing compounds. The extracts inhibited *Escherichia coli, Staphylococcus aureus, β-haemolytic Streptococcus* and *Klebsiella pneumonia*. There is an urgent need to establish the main bioactive agent and to experiment the use of the parts livestock feed.

KEYWORDS: Cola nitida, Cola rostada, polyphenols, antimicrobial, phytochemicals.

1 INTRODUCTION

As the world population continue to hit record high figures, some of the challenges facing the world include combating emerging infections and attaining food security. With many people living under a dollar per day especially in Sub-Saharan Africa, the endemicity of malnutrition is an understatement. The consequences of these are decreased resistance to diseases, lethargy, retarded growth, and death in extreme cases [1]. Not surprisingly, it has enhanced the search for so called medicinal plants and nutritional studies of these plants are also increasing. Interestingly, Nigeria is blessed with an abundance of such plants and as a result, alot of studies have been carried out on a variety of these medicinal plants [2], [3], [4], [5], [6], [7], [8]. *Cola nitida* and *Cola rostada* belong the family called *Sterculiaceae*, which consist of some 125 species of trees indigenous to the tropical rain forest of African region [9]. The fruit of *C. nitida* consist of an outer case called the pod husk and this contains about six to eight nuts individually wrapped up in a whitish smooth tissue called testa. Unlike the testa that is sweet, the seed is usually bitter and comes in three colours namely red, pink and white. *C. rostada* on the other hand is warty brown in colour with a sweet edible yellow pulp or mesocarp covered with a thin endocarp.

The importance of *C. nitida* cannot be overemphasized in Nigeria and some West African countries. From traditional marriage ceremonies to coronation of Kings or Chiefs, *C nitida* forms part of the menus. Caffeine, a component of Cola is an integral part of soft drinks and many pharmaceutical preparations. It also used as colouring agent and much of the studies available are carried on the seeds [1], [5], [10]. Accurate records do not exist regarding the exact tonnage of the seeds and pods of produced kola in Nigeria annually. However, the pods of *C. nitida* and seeds of C. rostada are usually discarded as waste. Studies have shown that *C. nitida* pods have good polyphenol content, can be used as fertilizers when dried and turned into ashed, and the alkaline rich ash can also be used in soap manufacturing [11]. Given the importance of these two commonly available Kolas, we decided to investigate the seeds and pods of these fruits for important phytochemical bases, basic nutrients and also check for antimicrobial activities against commonly encountered microorganisms.

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2 MATERIALS AND METHODS

SOURCE OF PLANT PARTS AND BACTERIAL CULTURE

The plant parts were sourced from Watt Market in Calabar, Cross River State, Nigeria. Already isolated and identified microorganisms were collected from the University of Calabar Teaching Hospital, Calabar, Cross River State. The Plants parts were identified at the University of Calabar Botanical Garden.

AQUEOUS EXTRACT

The weight of the various parts of the plants namely pod husk, seed and testa were accurately measured. The nuts and pods were cut into small pieces and dried by heating in an electric oven set at 105^{o}C for 48hours. The parts were grounded in a mechanical grinder into fine powder. About 100g of each powered plant sample were measured into four 250ml flask containing 100ml of distilled water. The solution was agitated and left to stand overnight. Each sample was then filtered using Whatman filter paper and the filtrate stored in a refrigerator.

ETHANOLIC EXTRACT

Exactly 100g of each powdered sample were percolated in 94% ethanol in clean sterilized bottles. These were allowed to stand for stand for some weeks. The extracts were then filtered using Whatman filter paper into crucibles of known weight and evaporated. The extract and the crucible were then weighed, placed in plastic universal bottles and stored at room temperature.

PHYTOCHEMICAL SCREENING OF PLANTS

The phytochemical bases screened for were alkaloids, saponins, tannins, cardiac glycosides, and phlobotannins using the methods of Sofowora [12]. Other included flavonoid, reducing compounds, mucilages, hydroxymethyl anthraquinones, anthraquinones, anthranoids and polyphenols according to methods previously reported [13], [14].

PROXIMATE COMPOSITION ANALYSIS

Proximate composition was carried out on all the samples by the standard methods laid down by the Association of Official Analytical Chemists (A.O.A.C) [15]. The analysis included moisture content, ash content, crude protein, lipid, crude fibre, carbohydrate, calorific value and mineral elements (Ca, Fe, Mn, Mg, Pb, Na, K, and P).

CAFFEINE OR AMMONIA EXTRACTION AND ESTIMATION OF TOXIC COMPONENTS

About 100g of the powdered kola samples were suspended in one litre of water and 100ml of ammonia solution. This was then shaken vigorously for 1hour and shaken with magnetic stirrer for 24hours. The mixture was filtered and the residue extracted once more with one litre of water and 100ml of ammonia solution. The filtrate was evaporated to dryness using rotator evaporator to obtain crude caffeine. Hydrocyanic acid was estimated using the methods of AOAC (1975) [15].

3 RESULTS

The results of the study are presented in the tables shown below. Table1 shows the results of the percentage content of the pod husks, seeds and testas of *Cola nitida* and *Cola rostada.* Values for *C. nitida* were comparatively higher in each of the kola parts. Table 2 shows the results of the proximate and elemental composition of both colas. The crude protein for example ranged from 8.6% to 11.91% while the carbohydrate content ranged from 65.49 to 86.2% for the two colas. The caloric values of both seeds were higher than those of the pods. However, the fibre contents of the pods were atleast four times those of the seeds. Table 3 shows the toxicant content of *Cola nitida* and *C. rostada*. The hydrocyanic acid content of the pod of *C. nitida* was more than that of pod of *C. rostada* while the reverse occurred in the case of the seeds. The highest amount of caffeine occurred in *C. nitida* seed followed by *C. rostada* pod.

Table 1: Percentage Content of Pod Husk, Seed and Testa of Freshly Harvested Fruits of C. nitida and C. rostada

Components	*C. nitida (%)**	*C. rostada*
Pod Husk	47.79	26.68
Seed	38.66	24.42
Testa	13.55	-
Mesocarp	-	47.30
Endocarp	-	1.60

Values were based on ten determinations. This indicates that the edible portions(seed) account for almost half of the weight of C. rostada while the Pod Husk for C. nitida did weigh more than the seed.

Table 2: Proximate and Elemental Composition of the Cola Parts

Components	*C. nitida*		*C. rostada*	
	Pod	Seed	Pod	Seed
Moisture	81.80	54.2	81.7	56.3
Crude Protein	11.91	8.40	9.63	10.5
Lipid(ether extract)	0.80	2.00	1.2	2.00
Crude fibre	15.80	2.00	14.60	3.60
Ash	6.0	2.20	5.0	2.00
Carbohydrate	65.49	86.20	69.58	81.90
Caloric value	316.8	388.20	327.62	387.60
Sulphur	1.01	0.85	1.43	0.61
Nitrogen	2.50	1.60	3.00	1.80
Phosphorus	0.36	0.24	0.61	0.38
Potassium	6.50	1.00	7.80	1.46
Calcium	1.12	0.16	0.95	0.18
Magnesium	0.45	0.33	1.10	0.46
Sodium	1.12	0.68	1.65	0.79
Cobalt	0.01*	0.01	0.01	0.01
Manganese	0.60	0.40	0.66	0.48
Iron	1.81	1.00	1.68	1.06
Cadmium	0.01	0.01	0.01	0.01
Lead	0.02	0.02	0.03	0.02

**Cobalt, Manganese, Iron, Cadmium, and lead are expressed in ppm. While sulphur, nitrogen, phosphorus, potassium, calcium, magnesium and sodium are expressed in % mg/100g.*

Table 3: Toxicant Composition of the Colas (% mg)

Toxic Components	*CN Pod	CN Seed	*CR Pod	CR Seed
Hydrocyanic acid	4.00	0.98	1.19	2.16
Tannins	491	979	198	125
Oxalic acid	9.24	15.62	11.22	1.10
Phytic acid	1.39	3.06	1.37	1.44

**CN, C. nitida, CR, C. rostada*

The result of the phytochemical screening is presented in table 4. The results indicate the presence of alkaloids, saponins, tannins, reducing compounds and polyphenols whereas phlobotannins, anthranoids and anthraquinones were absent in the aqueous extracts of the pods and seeds respectively. In the ethanolic extract, only tannins, polyphenols and reducing compounds were present in all pods and seeds. Table 5 shows the result of the antimicrobial test of the extract. The aqueous extract of the pods and seeds of both plants completely inhibited the growth of some organisms but not *Proteus mirabilis* and *Neisseria gonorrhoeae*. *S. aureus* was completely inhibited by the aqueous and ethanolic extract of the pods and the seeds of both colas. *E. coli* and □-haemolytic *Streptococcus* were also inhibited by the crude ethanolic extract of the seeds of both *C. nitida* and *C. rostada* but only by the pods. *K. pneumonia* was inhibited only by the pod and seed of *C. rostada.*

Table 4: Phytochemical Composition of Aqueous and Ethanolic Extract of Cola Parts

Phytochemical Components	Aqueous Extract CN Pod	CN Seed	CR Pods	Ethanolic Extract CR Seed	CN Pod	CN seeds	CR Pod	CR Seed
Alkaloids	+	+	+	+	-	+	-	+
Saponins	+	+	+	+	-	+	-	+
Tannins	+	+	+	+	+	+	+	+
Cardiac glycosides	-	+	-	+	-	+	-	+
Reducing compound	+	+	+	+	+	+	+	+
Polyphenol	+	+	+	+	+	+	+	+
Hydroxymethyl anthraquinones	-	+	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-
Anthronoids	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-

CN, Cola nitida, CR, Cola rostada

Table 5: Antimicrobial Susceptibility of Crude Aqueous and Ethanolic Extracts of Cola Parts on Pathogenic Bacteria (Zone of Inhibition- mm)

Organisms	Aqueous CN Pod	CN Seed	CR Pod	CR Seed	Ethanolic CN Pod	CN Seed	CR Pod	CR Seed
P.mirabilis	-	-	-	-	-	-	-	-
E. Coli	22.0±0.57	-	22.0±0.57	-	23.0±0.57	-	23.0±0.57	-
S. aureus	21.6±0.19	21.6±0.43	21.6±0.19	21.6±0.19	22.6 ±0.84	22.6±0.19	22.0±0.57	22.0±0.57
haemolytic Streptococcus	22.3±0.19	23.0±0.57	22.0±0.57	-	21.6±0.43	-	25.0±0.57	-
K. pneumonia	-	-	-	-	23.3±1.4	23.0±0.37	-	-
N.gonorrhoeae	-	-	-	-	-	-	-	-

CN, Cola nitida, CR, Cola rostada

4 DISCUSSION

One of the most studied kola is C. nitida [16], [17]. Previous studies have revealed the chemical and elemental composition of the seed of *C. nitida* but nothing was done on *C. rostada*. An average moisture content of 54% for the seeds of C nitida has been reported [16] whereas our study reveals a similar content of 56%. Equally present in good quantity was carbohydrate, protein and crude fibre. This abundance of essential nutrients suggests that the pods of C nitida and seeds C. rostada can be converted into feeds for livestock instead of wasting them as it is the current practice. However, toxicant analysis revealed that the pods and seeds of the two fruits particularly C. nitida had had very high content of tannin and low in hydrocyanic acid. It has been suggested that fermentation using various yeasts could greatly reduce the tannin content rendering this plant part fir for use as feed supplement [18]. Hydrocyanic acid has been shown to be taken care of by microorganisms especially in fermented foods such as garri [19], [20]. Other interesting feature of these plant parts is the elemental composition the plants had very low content of heavy metals such as cobalt cadmium and lead. Other elements though not high in concentration are element useful to the body.

The presence of phytochemical components that exhibit inhibitory activity on microbes is an indication that such plants may contain bioactive components that are useful for preparation of pharmaceuticals [20]. An attempt to elucidate the chemical nature of the bioactive substance in a close species *Garcinia kola* revealed a polyphenolic compound [22] and very abundance in our samples. Another study has shown that this polyphenolic bioactive compound has inhibitory effect on key enzymes - □-amylase and □-glucosidase activities linked to type 2 diabetes in rat models [7]. The crude aqueous and ethanolic extracts of the plants parts inhibited S. aureus (table 5). The zones of inhibition were on the average better than that previously reported for *E. coli* and *S. aureus* [4], [8]. However, *E. coli*, and □-haemolytic Streptococcus were inhibited by only the pods of the two kolas and not the seeds. The latter microorganism also recorded the highest inhibition in our study. None of the extracts were able to inhibit the growth of *N. gonorrhoeae* and *P. mirabilis.* It was also observed that the ethanolic extracts of pods and seeds of *C. nitida* inhibited *K. pneumoniae*. C. nitida did show a better antimicrobial activity compared to its counterpart in our study.

5 CONCLUSION

It is proposed that in Nigeria about 11,000 hectares of *C. nitida* are planted both in unregulated areas and in controlled orchards [23]. Given the rich nutritional base of the plant parts and current practice of treating the pods and seeds of C. nitida and C. rostada as waste and dumping them away annually, we propose that more attention be paid to planting these valuable crops and that the pods and seeds be experimented as feed supplement for livestock such as goats and sheep. Furthermore, more studies should be carried out to elucidate the exact chemical nature of the bioactive agents responsible for the inhibition of commonly encountered microorganisms.

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