Global Scientific JOURNALS

GSJ: Volume 8, Issue 10, October 2020, Online: ISSN 2320-9186
www.globalscientificjournal.com

Evaluation of Phytochemical and Enzyme Activities of fresh Kola (*C. nitida vent*) of varying weights (g) and colours.

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Abstract

Cola belongs to the family Sterculiaceae. Fresh *C. nitida* nuts of different weights (1-10g, 11-20g and above 21g) and colours (pink, red and white) were obtained from Cocoa Research Institute of Nigeria, Ibadan, Oyo State. These Nuts were oven dried at 70^0C for 2 days, ground into powder for phytochemical and enzymatic analysis. Data were subjected to Analysis of variance. Increased phytochemical content from the smallest nut weight to the largest nut weight with the preponderance of anthraquinone ranged between 3.51g/100g to 6.38g/100g and the lowest mean value was obtained in alkaloids ranged between 1.44g/100g to 2.06g/100g. Red *C. nitida* nut had the highest mean value in tannin (4.22g/100g). The pink *C. nitida* nut recorded the highest mean values in alkaloid and saponin and was significant when compared to the red and white nuts. Similar result was obtained for chemical composition with increased values from the smallest nut weight to the biggest nut weight ranged between 3.32g/100g to 5.42g/100g for caffeine, 1.65 g/100g to 2.28g/100g for theobromine, 3.44g/100g to 4.78g/100g for kolatin, 1.57g/100g to 2.76g/100g for polyphenol and 4.21 to 5.75 for vitamin C. respectively. Increased enzymatic activities were observed from the smallest nut weight to the biggest nut weight with the preponderance of total (α and β) amylase activity ranged between 0.137mg maltose/min/g protein to 0.148mg maltose/min/g protein, followed by catalase, 0.136mg glucose/min/g protein to 0.145mg glucose/min/g protein and the lowest mean values was obtained for proteinase, 0.113mg tyrosine/min/g protein to 0.121mg tyrosine/min/g protein.. Similar results were obtained for the different colours. In conclusion, *C. nitida* nut >21g contain high level of phytochemical and enzymatic activities compared to medium and small nuts.

Keywords: phytochemicals, *C. nitida* nut, Enzymes activities, chemical composition

Introduction

In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs. Thus a search for new drugs with better and cheaper substitutes from plant origin is a natural choice. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al*., 2005).

Cola nitida (the kola nut) also known as cola, goro nut, cola seed is an important commercial and economic crop for many West African countries (Lovejoy, 1980) and Central Africa (Van Eijnatten, 1969). There are about twenty five species of the genus cola but only five or six species produce edible seeds commonly called 'nuts'. These are *Cola nitida* (vent), *Cola acuminata* Schott and Endl., *Cola vertillata* Thorn, *C. anomala* and *C. ballay* (Russell, 1955). However, only two species; *C. nitida* and *acuminata* are cultivated as plantation crops (Quarcoo, 1973; Daramola, 1978).

For many decades, kola has served and still serves as the main cash crop for many farmers in West Africa. Kola nut has been an important article of international trade in many parts of Africa (Nzekwu, 1961). Kola nuts are used as a masticatory and the only stimulant allowed to Muslims. For this reason there is a heavy trade of kola from the humid southern regions to the northern arid parts of West Africa. It is a very special and important

item used in social and ceremonial activities by Africans, especially those of the Sahelian zones. Earlier in the century, demand for kola nuts in the world markets for the manufacture of kola flavoured drinks, alcoholic beverages, candy and nutritional supplements (Beattie, 1970; Ogutuga, 1975) existed.

Due to the economic and medicinal value of kola both locally and international, this work was therefore carried out to determine the level of polyphenol, phytochemical and enzymatic activities of fresh *Cola nitida* nut which is one of the ways in assessing kola quality

MATERIALS AND METHODS

This study was carried out at Cocoa Research Institute of Nigeria (CRIN), Idi -Ayunre, Ibadan, Oyo State, Nigeria

Sample collection

Kola samples were collected from Cocoa Research Institute of Nigeria

Polyphenol, phytochemical and enzymes analyses of *C. nitida*: Fresh *C. nitida* nuts with three different sizes and colours collected from CRIN were used for this study. The nuts were classified into weights as follows: 1-10g, 11-20g and above 21g and into colours: red, pink and white. The nuts were crushed separately into smaller particle sizes using perforated grater and stored in a capped container until they were needed for analysis.

Determination of alkaloids

This was done by the alkaline precipitation gravimetric method described by Harborne (1973). A measure weight of the sample was dispersed in 10 % acetic acid solution in ethanol to form a ratio of 1:10 (10 %). The mixture was allowed to stand for 4h at 28^{o}C. It was later filtered via Whatman No 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of conc. Aqueous NH_4OH until the alkaloid was precipitated. The alkaloid precipitated was received in a weigh filter paper wash with 1% ammonium solution dried in the oven at 80^{o}C. Alkaloid content was calculated and express as a percentage of the weight of sample analyze.

Flavonoid

This was done according to the method of Harborne (1973). Five gram of the sample was boiled in 50 ml of 2M HCl solution for 30 mins under reflux. It was allowed to cool and then filtered through Whatman No 42 filter paper. A measure volume of the extract was treated with equal volume of ethyl acetate starting with drop. The flavonoid precipitated was recovered by filtration using weigh filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

Tannin

Tannin content was determined by the Folis- Dennis colorimetric method described by Kirk and Sawyer (1998). Five gram of sample was dispersed in 500 ml of distilled water and shaken. The mixture was allowed to stand for 30 mins at 28^{o}C before it was filtered through Whatman No 42 grade of filter paper. 2 ml of the extract was dispersed into a 500 ml volumetric flask. Similarly, 2 ml standard tannin solution (tannic acid) and 2 ml of distilled water was put in separate volumetric flask to serve as the standard; and reagent was added to each of the flasks, and the 2.5 ml of saturated $NaCO_3$ solution was added. The content of each flask was made up to 50 ml with distill water and allowed to incubate at 28^{o}C for 90 mins. Their respective absorbance was measured in a spectrophotometer at 260 nm using the reagent blank to calibrate the instrument to zero

Saponin

Quantitative determination of saponin was done according to Obadoni and Ochuko (2001). Twenty gram of each powdered sample was added to 100 ml of 20 % aqueous ethanol and kept in a shaker for 30 mins. The sample was heated over water bath for 4 h at 55^{o}C. The mixture was then filtered and the residue re- extracted with another 200 ml of 20 % aqueous ethanol. The combined extract was reduced to approximately 40 ml over bath at 90^{o}C. The concentrate was transferred into 250 ml separatory funnel, extracted twice with 20 ml diethyl ether. Ether layer will be discarded while aqueous layer was retained and 60 ml n- butanol extract was washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath and after

evaporation; the sample was dried in an oven at 40^{o}C to a constant weight. The saponin content was calculated as percentage of the initial weight of sample taken

Enzyme determinations

Cellulase activity: The enzyme extract was prepared by grinding 1g of nut with 1/10 M dibasic sodium phosphate (K_2HPO_4) in a mortar maintained at 5^{o}C with crushed ice (Norkrans, 1957). The ensuing suspension was centrifuged at 18,000 g for 30 mins at 2^{o}C using the M.SE ultra-high speed centrifuge. To 1 ml of the supernatant was added 1 ml of 1 % carboxymethyl cellulose in 0.05 M phosphate buffer (pH 5.0) and the mixture was allowed to stand for 1 h at 30^{o}C (Singh and Kunene, 1980). The enzyme action was stopped with 3, 5 - dinitrosalicylic acid (DNSA) reagent and the amount of reducing sugar formed was determined by taking the absorbance at 540 nm against a blank containing 1ml of boiled enzyme extract which was similarly treated. (Koelin, 1977).

Total amylase (α and β activity): Enzyme extract was prepared by grinding 1g of nuts with sodium acetate buffer (pH 5.0) in a mortar maintained at 5^{o}C with crushed ice and the extract centrifuge at 18,000 g for 30 mins at 2^{o}C. 1 ml of the supernatant was added 1ml of 1 % soluble starch in 1/10 M sodium acetate buffer and the mixture was incubated at 27^{o}C for 1 h. The enzyme action was stopped with DNSA reagent and the quantity of reducing sugar formed was determined by taking the absorbance at 540 nm against a blank containing 1 ml of boiled enzyme extract treated similarly (Swain and Dekker, 1966).

Proteinase activity: Enzyme extract was prepared in a manner similar to total amylase activity except that 0.05 M sodium phosphate buffer (pH 6.0) was used as the extracting buffer proteinase activity in the enzyme was determined using the Lowry folin –ciocalteu method of Mcdonald *et al.* (1965).

Lipase activity: Enzyme extract was obtained in the same way for total amylase activity. Lipase activity in the enzyme extract was determined using the method of Young *et al*. (1977).

Polyphenol oxidase activity total polyphenol, and ascorbic acid was determined by the method described by Luigi (1968) as modified by Prince and Butler (1977)

Ascorbic acid content:
The determination of ascorbic acid was carried out using the indophenol method described by (Association Official Analytical Chemists, (1984). Ten gram of fresh kola nuts was weighed into mortar and 48ml metaphosphoric acid was added. The mixture was stirred for about 20 mins and rapidly filtered using a suction pump and Buchner funnel. 10 ml of the filtrate was titrated to the end point (change from blue to a permanent pink colour) with the standardized 2, 6-dichlorophenol-indophenol solution. The titration was repeated in triplicates and blank determinations was also carried out following the above procedure but using 10 ml of mataphosphoric acetic acid instead of the filtrate (AOAC, 1984).

Caffeine determination

Caffeine content was determined according to Irgolic *et al.* (1982) methods. Two samples of grated kola nuts were put into a Round Bottom Flask and 300 ml of distilled water was added to each. The mouth of the flask was covered with condenser and then connected to close but running tap and each of the flasks was placed on electric heater. As soon as the content began to boil the close tap was opened to drain the water and the set was allowed to stand for one hour. As the content was boiling, the refluxing system was turned on and the reflux sieved out grated Kola (with 0.1 mm and 0.2 mm sieve) into 200 ml beaker. The residue was discarded and the filtrate was retained and placed in ice block for 15 mins. Thereafter, 100 ml of the filtrate was placed in a 250 ml separatory funnel and 120 ml of chloroform was added gradually. The corked separatory funnel was shaken until the chloroform-water interface was established, and after 50 mins clear solution was formed into which caffeine dissolved in the chloroform. It was later put into 50 ml beaker and chloroform evaporated over a water bath. The weight of the resultant yellowish white caffeine crystals was taken on mettler P-165 electric balance

Results and discussion

Phytochemical screening showed a common trend of increasing order from the smallest nut weight to the biggest nut weight assayed with the preponderance of anthraquinone ranged between 3.51g/100g to 6.38 g/100g, followed by tannin, 2.75 g/100g to 4.42 g/100 g while alkaloid recorded the lowest mean values ranged between 1.44g/100g to 2.06 g/100 g (Table 1). Pink *C. nitida* nut had the highest mean values of 4.01g/100g and 1.87g/100 g for saponin and alkaloid, followed by red nuts with values 4.22g/100g for saponin, 3.01g/100g for flavonoid and 4.22 g/100g for anthraquinone while the white nuts recorded lowest mean values ranged from 2.96g/100g for saponin and 1.79g/100g for flavonoid respectively (Table 2). The presence of secondary metabolites in the kola nuts could be responsible for its antioxidant activity. For example, flavonoid and other phenolic constituents have been shown to play a preventive role in the development of cancer and heart diseases. Potential sources of antioxidant compound have been searched in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, bark and roots, spices and herbs and crude plant drugs (Wang *et al*., 2000; Pourmorad *et al*., 2006; Kumaran and Karunakaran, 2007). In addition, phenols are widely used in the manufacture of resins, plastics, insecticides, explosives, dyes and detergents as a raw material for the production of medicinal drugs such as aspirin (Michael, 2008). Therefore, *C. nitida* can then be of economic importance in these aforementioned areas.

Table 1: Quantitative phytochemical content (g/100g) of fresh *Cola nitida* nuts of different weights

Nuts weights(g)	Alkaloids	Tannin	Saponin	Flavonoid	Anthraquinone
>21g	2.02[a]	4.42[a]	4.25[a]	3.03[a]	6.38[a]
11-20	1.84[b]	3.97[b]	3.68[b]	2.32[b]	5.38[b]
1-10	1.44[c]	2.75[c]	2.66[c]	2.04[c]	3.51[c]

Means followed by the same letters on the same columns are not significantly different at 5% probability level using Duncan Multiple Range Test.

Table 2: Quantitative phytochemical content (g/100g) of fresh *Cola nitida* nut of different Colours

Nuts colours	Alkaloid	Saponin	Anthraquinone	Flavonoid	Tannin
Pink (>21g)	1.87[a]	4.01[a]	5.67[a]	2.58[b]	3.23[c]
Red (>21g)	1.68[b]	3.61[b]	5.17[b]	3.01[a]	4.22[a]
White(>21g)	1.79[a]	2.96[c]	4.43c	1.79[c]	3.69[b]

Means followed by the same letters on the same columns are not significantly different at 5% probability level using Duncan Multiple Range Test.

The chemical composition of fresh *C. nitida* nut showed a common trend of increase from the smallest nut weight to the biggest nut and the result is highly significant with vitamin C having the highest value which ranged from 4.21 to 5.75. This was followed by caffeine ranged between 3.32g/100g to 5.42g/100g and theobromine the least ranged from 1.65g/100g to 2.28g/100g (Table 3). The chemical composition of different colours shows that red nuts above 21g had the highest mean values in all the chemical content assayed with the preponderance of vitamin C when compared to pink and white nuts. This was followed by pink nuts and the white nuts the lowest. (Table 4). These values were higher than the 1.5% obtained by Ogutuga (1975) and Jaiyeola (2001) for *C. nitida*. Caffeine occurs partly free and partly combined in an unstable complex known as

kolatin (tannin) and caffeine glycosides in kola. Pure caffeine is colourless and has a distinctively bitter taste at the temperature, pH and salt concentrations normally encountered in food processing (Graham, 1978). It is also known to produce a variety of biological effects. Thus, caffeine is widely used for its stimulant properties in dietary beverages. Furthermore, definitive tests have shown that caffeine is not adaptive as regular consumption does not diminish its stimulant effects and its consumption in high amounts may become toxic (Graham, 1978). The percentage of kolanin in kola nuts is usually 5 to 10 % and is made up of catechol and epicatechol. This complex oxidizes and hydrolyses to form kola-red and free caffeine under the influence of enzymes when the nuts are drying out (Adeyeye and Ayejuyo, 1994). In addition kola nuts in this study contain considerable amount of theobromine which ranged from 1.65 g/100 g for the smallest nut weight to 2.28 g/100 g for the biggest nut weight which is contrary to (Adeyeye and Ayejuyo, 1994) who reported that theobromine contain very small quantities of (0.02 to 0.08 %) theobromine (3,7-dimethylxanthine) and theophylline (1, 3-dimethylxanthine).

Table 3: Chemical composition (g/100g) of fresh *Cola nitida* nuts at different sizes

Nuts weights (g)	Caffeine	Theobromine	Kolatin	Phenol	Vitamin c
21-40	5.42[a]	2.28[a]	4.78[a]	2.76[a]	5.75[a]
11-20	4.50[b]	1.97[b]	4.27[b]	2.28[b]	4.80[b]
1-10	3.32[c]	1.65[c]	3.44[c]	1.57[c]	4.21[c]

Means followed by the same letter on the same columns are not significantly different at 5% probability level using Duncan Multiple Range Test

Table 4: Chemical Compositions (g/100g) of fresh *Cola nitida* nuts at different Colours

Colours nut	Caffeine	Theobromine	Kolatin	Polyphenol	Vitamin C
Pink (>21g)	4.83[a]	2.13[b]	4.55[b]	1.45[c]	4.84[b]
Red (>21g)	4.46[b]	2.29[a]	4.69[a]	3.61[a]	5.55[a]
White(>21g)	3.97[c]	1.47[c]	3.25[c]	1.55[b]	4.36[c]

Means of the same letters on the same columns are not significant different at 5% probability level using Duncan Multiple Range Test

Enzymes activities increased from the smallest nut weight to the biggest nut weight of fresh *C. nitida* with the preponderance of total amylase activity with values ranged from 0.137mg glucose/min/g protein to 0.148mg glucose/min/g protein, followed by catalase activity with values ranged from 0.136mg glucose/min/g protein to 0.145mg glucose/min/g protein. Lowest mean value of enzyme activity was obtained in proteinase ranged from 0.113mg tyrosine/min/g protein to 0.121mg tyrosine/min/g protein. Enzymes activities of varying colours above 21g showed that the red nuts had the highest mean values in all the enzymes analyzed with the preponderance of total amylase activity with values ranged from 0.131mg glucose/min/g protein for pink to 0.149mg glucose/min/g protein for red, followed by catalase activity with value 0.130mg glucose/min/g protein for pink to 0.146 mg glucose/min/g protein for red and the lowest mean value was obtained in proteinase activity with value 0.115 mg tyrosine/min/g protein for pink to 0.118 mg tyrosine/min/g protein for red and white. Purse glove (1991) reported values of polyphenol in the range of 0.8 % to 1.3 % for garcina kola and 2.5 % to 3.0 % for the varieties of *Cola nitida*. Work carried out by Ducksworth and Coleman (1970) showed the

white cultivars of most crop products such as bitter kola and kola nuts lack carotene, a polyphenol, which is responsible for the pigmentation, noticed in *Cola nitida* especially the red cultivar. The relatively considerable values of polyphenol in this study may therefore explain the incidence of enzymatic browning in Nigeria kola nuts. The levels of polyphenol also vary from variety to variety with the highest value occurring in the red cultivar of *Cola nitida* and the least obtained was pink. A kola nut occupies a unique place amongst West Africans where it is widely consumed by them. An interesting occurrence takes place when kola nuts are half eaten or handled in a manner that exposes their tissues; brown particles begin to appear with time, which stain the teeth of notable consumers. This principle is referred to as "browning" Browning is attributed to the oxidation of phenolic compound present in the plant product. This is catalyzed by polyphenol oxidase. This enzyme utilizes molecular oxygen in producing quinines and melanin, which on interaction with other constituents yield brown pigments (Mayer and Havel, 1979).

Table 5: Enzyme activities of fresh *C. nitida* nuts of different sizes

Nut weights (g)	Catalase mg glucose/min/ g protein	Polyphenol oxidase mg glucose/min /g protein	Total (α & β) amylase mg maltose/min/ g protein	Proteinase mg tyrosine/min/ g protein	Glucose-6-phoshatase mg glucose/min/g protein	Lipase ml 0.02NaOH/ min/g protein	Cellulase mg glucose/min/g protein
21-40	0.145[a]	0.140[a]	0.148[a]	0.121[a]	0.123[a]	0.134[a]	0.143[a]
11-20	0.140[b]	0.132[b]	0.145[b]	0.117[b]	0.119b	0.121[b]	0.128[b]
1-10	0.136[c]	0.130[c]	0.137[c]	0.113[c]	0.118[c]	0.119[c]	0.124[c]

Means followed by the same letters on the same columns are not significantly different at 5% probability level using Duncan Multiple Range Test

Table 6: Enzyme activities of fresh *C. nitida* nuts of different sizes

Nut colours	Catalase mg glucose/min/g protein	Polyphenol oxidase mg glucose/min /g protein	Total (α & β) amylase mg maltose/min/ g protein	Proteinase mg tyrosine/min/ g protein	Glucose-6-phoshatase mg glucose/min/g protein	Lipase ml 0.02NaOH/ min/g protein	Cellulase mg glucose/min/g protein
Pink (>21g)	0.130[b]	0.126[b]	0.131[b]	0.115[b]	0.116[a]	0.116[b]	0.121[a]
Red (>21g)	0.146[a]	0.139[a]	0.149[a]	0.118[a]	0.121[a]	0.122[a]	0.147[a]
White (>21g)	0.145[a]	0.139[a]	0.149[a]	0.118[a]	0.137[a]	0.122[a]	0.128[a]

Means followed by the same letters on the same columns are not significantly different at 5% probability level using Duncan Multiple Range Test

Conclusion

The present study showed that kola nuts contain some active ingredients which can be useful in the pharmaceutical and medical science because of the presence of secondary metabolites to make vaccine and supplements that can prevent diseases. Considerable amount of alkaloid and flavonoid content suggest their antioxidant potentials and justifies their therapeutic actions, which could be used in drug formulation. It can be useful also in various manufacturing industries as raw material. This study also showed that *C. nitida* nut contains considerable amount of polyphenol, responsible for the astringency and bitterness of the nuts.

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