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## **Caffeine and Phenolic Compounds in *Cola nitida* (Vent.) Schott and Endl and *Garcinia kola* Heckel Grown in Côte d'Ivoire**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author YN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors FA, FN, OC, AA, GHB managed the analyses of the study and literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Kola nuts are usually eaten fresh and represent a substantial source of income for many households and certain public authorities. Their importance lies in their content of some secondary metabolites of interest used in industries for the production of energy drinks and pharmaceuticals. The aim of this study is to reveal and determine the content of

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polyphenols (flavonoids in particular) and caffeine in nuts from Côte d'Ivoire. According to their phenotypical characteristics, four lots of nuts were collected (Red *Cola nitida* (RCN); White *Cola nitida* (WCN); Purple *Cola nitida* (PCN) and *Garcinia kola* (WGK)). After drying, different extractions were carried out using several solvents: water, acidified water (0.01N citric acid), methanol, ethanol, acetone 60% and methanol/acetic acid 1% solution. Phytochemical screening showed that kola nuts contain antioxidants such as flavonoids, tannins and alkaloids in varying proportions according to the species. Spectrophotometric analysis revealed that total polyphenols and flavonoids are important in *C. nitida* nuts compared to those of *G. kola*. Phenolic contents are  $26.76 \pm 0.54$ ;  $23.08 \pm 1.06$ ;  $17.06 \pm 1.03$  and  $14.90 \pm 0.64$  mg/g FW for WCN, RCN, PCN and WGK respectively. As for flavonoids, levels are  $803.03 \pm 14.48$ ,  $697.13 \pm 12.76$ ,  $647.76 \pm 21.16$  and  $355.74 \pm 17.03$  mg/kg FW for WCN, RCN, PCN and WGK respectively. It noted that acetone 60% is the efficient solvent for extraction. Statistical analyzes indicate the significant influence at  $P=.05$  of solvent and nuts morphotype on the level of extractable for total polyphenols ( $P=.000000$ ) and flavonoids ( $P=.000000$ ) respectively. Concerning caffeine, reversed-phase HPLC analysis indicates that concentrations are higher in *C. nitida* extracts with a slight predominance for RCN ( $10812.5 \pm 6.27$  mg/kg FW). We note a small amount of this metabolite ( $80.08 \pm 2.91$  mg/kg) in *G. kola*. The mean intakes of nutrients are 3.99 and 1217.13 mg of polyphenol/day for an equivalent daily intake of 0.6 g (cola amount consumed per day in West Africa) and 183 g (amount of fruit consumed per day). About caffeine, intakes are 4.31 and 1313.05 mg/day for 0.6 and 183 g respectively. These findings indicate that kola seeds are enriched in important compound and can be used as a possible source of antioxidant for African population's customs and European industries.

**Keywords:** *Cola nitida*; *Garcinia kola*; Kola nuts; phenolic compounds; caffeine; nutrient intake; Côte d'Ivoire.

## 1. INTRODUCTION

The genus *Cola* (*Sterculiaceae*) comprises about 140 species [1] greatly widespread in the Central and Western parts of Africa [2,3]. In Côte d'Ivoire, the most commonly met species were *Cola acuminata* (P. Beauv) Schott & Endl. and *Cola nitida* (Vent.) Schott & Endl [4]. But, only *C. nitida* is locally cultivated and widely consumed [4]. They were generally exploited for their edible fruits containing seeds or nuts [5] of economic importance. 100,000 t of *C. nitida* nuts were annually locally produced allowing Côte d'Ivoire to be one of the first producers and world exporters [4]. *C. nitida* nuts are also received great interest due to their social significance [6] and pharmacological properties such as physical and intellectual stimulant, vomiting control in pregnant women [7,8], hunger and thirst counteracting effects [9,10]. Besides its strong stimulants effects, the nuts present many other beneficial biological activities such as antidiarrheal, antidyenteric, lipolytical and analeptic effect [11].

The nuts of *C. nitida* were sometimes substituted by *Garcinia kola* Heckel (Guttifereae) nuts [5] locally named "small cola". These nuts taste was bitter astringent when chewed [7,1] and were used in local medicine to relieve cough, colics, chest, cold, headaches to manage diabetes and hepatotoxic diseases [12,5]. They were usually used by people to enhance the flavor of some beverages due to their astringent and aphrodisiac properties [13,14].

Studies have been conducted on the nuts of *C. nitida* and *G. kola* in some African countries. They showed a variability of alkaloid and polyphenol content of nuts according to their

phenotypical type [15] and harvest areas [1]. Nutrient intake of kola nuts vary from one species to another. Recently, the estimation of the consumption of polyphenols using the database Phenol Explorer showed that the average intake of polyphenols could be estimated at a value between 1 and 1.2 g/day [16] and the caffeine between 200 and 400 mg/day according to Health Canada [17], the daily consumption of cola is 0.6 g/day for a 60 kg adult reflecting a low nutrient intake.

The therapeutic properties previously reported on kola nuts extracts were attributed to the intrinsic bioactive compounds among what caffeine and polyphenols. Caffeine and polyphenols are naturally present in many foods beverages [18]. Moreover, various pharmaceutical products contain synthetic caffeine and polyphenol in combination with other drugs [18]. But the problem of toxicity usually revealed by the use of synthetic drugs provoked a growing interest for natural sources of drugs such as caffeine and polyphenol. Kola nuts were industrially known to possess strong caffeine content (1.5-3.8%) and polyphenols such as flavonoids and tannins depending on the variety of the nut characterized [15] and therefore were industrially sought as source of this bioactive compound. Although Côte d'Ivoire was the important producer and exporter of kola nuts in the world [4], no data was unfortunately available about their qualitative and quantitative determination. This situation is not without adverse consequences for our country in traceability terms and promoting a specific label behind Côte d'Ivoire. For this reason, this study was needful. The aim of this work was firstly to revealed and quantify polyphenols and caffeine in *C. nitida* and *G. kola* harvested in Côte d'Ivoire, secondly show the effect of solvents on the extractable nuts, that in order to reduce or ignore the expensive and dangerous solvents for human health in future studies and finally assess the nutrient intake.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

Four phenotypes of *C. nitida* (3) and *G. kola* (1) fresh nuts were harvested and collected from the growing areas of the region of Agboville (78 km far from Abidjan) Côte d'Ivoire. As shown in Table 1, the fresh material samples RCN, PCN, WCN, WGK were organized and then sent to laboratory. The various nuts were cut into small pieces with a knife and then dried at room temperature (30±2°C). After drying, samples were then milled using an electric blender (IKA M20, Germany) and stored in polybags at room temperature.

**Table 1. Sampling of kola nuts of *Cola nitida* and *Garcinia kola***

Sample identification	Fresh nuts color	Kolanuts species	Harvesting month
RCN <sup>1</sup>	Red	<i>C. nitida</i>	December
PCN <sup>2</sup>	Purple	<i>C. nitida</i>	December
WCN <sup>3</sup>	White	<i>C. nitida</i>	January
WGK <sup>4</sup>	white	<i>G. kola</i>	january

<sup>1</sup> RCN: Red *C. nitida*

<sup>2</sup> PCN : Purple *C. nitida*

<sup>3</sup> WCN: White *C. nitida*

<sup>4</sup> WGK: White *G. kola*

## **2.2 Chemical and Standard**

All reagents and solvents were of analytical grade. Ethanol and methanol were purchased from Carlo Erba (Spain), acetic acid from Panreac (USA), Folin-Ciocalteu reagent from Panreac (USA), gallic acid and quercetin, Sigma-Aldrich (Germany), sodium carbonate and chloride aluminium, Merck (Germany), sodium hydroxide, Sharlau (Spain), acetone from ACI (Côte d'Ivoire) and citric acid from Riedel-de-Haen (Germany). Caffeine was obtained from Sigma Aldrich (USA). Water was purified by a Milli-Q water purification system.

## **2.3 Drying Kinetics**

The samples are dried to be weighed daily until obtaining constant weight translating the end of drying. The drying was carried out at room temperature ( $30\pm 2^\circ\text{C}$ ) in a room safe from light rays. The drying kinetics of different nuts were then performed (Weight (%) = f (drying time (days))).

## **2.4 Phytochemical Analysis**

The phytochemical analysis of extracts of various kolanuts extract were carried out according to the standard method of Trease and Evans [19]. Various reagents and tests were used:  $\text{FeCl}_3$  for tannin, frothing test for saponin, Dragendorff and Mayer reagents for alkaloids, magnesium chip and HCl for flavonoids. The presence of a bioactive compound was indicated by a color change and a precipitate.

## **2.5 Extraction of Polyphenols**

Total polyphenols extraction was performed according two methods:

### **2.5.1 First extraction (solvents: water, acidified water, methanol and ethanol)**

An aliquot of 1 g of dry nut obtained using a blinder (IKA M20, Germany) was left to soak for 30 minutes under ultrasound (JP Selecta Spain) in 200 mL of solvent (water, acidified water (citric acid, 0.01N), ethanol) at a solid/liquid ratio (w/v)  $R = 1/200$ . Macerate obtained is filtered to obtain crude extracts and diluted if necessary before different assays [20].

### **2.5.2 Second extraction (acetone 60%)**

A weight of 500 mg of dry cola seed were ground in acetone/water 60:40 (v/v). The phenolic compounds were extracted by constantly shaking the blend samples on ice three times with 50 mL of 60% aqueous acetone. Each shaking was followed by centrifugation at room temperature at 5000 rpm for 15 min. The three supernatants were combined in a flask containing 2 mL of glacial acetic acid. The acetone was removed by rotary evaporation under a partial vacuum at  $40^\circ\text{C}$ . The aqueous phase obtained was adjusted to 100 mL with Milli-Q Plus water in a volumetric flask. The total content in polyphenolic compounds was analyzed from this aqueous phase [1].

## **2.6 Polyphenols Levels**

Total polyphenols were determined using the method described by Singleton and Rossi [21], as amended by Wood et al. [22]. A volume of 2.5 mL of Folin-Ciocalteu diluted (1/10) was

added to 30  $\mu\text{L}$  of extract. The mixture was held for 2 minutes in the dark at room temperature, and then 2 mL of calcium carbonate ( $75 \text{ g.L}^{-1}$ ) were added. The mixture was placed for 15 minutes in a water bath at  $50^\circ\text{C}$ , and then cooled quickly. The absorbance was measured at 760 nm with distilled water as blank. A calibration curve was performed with gallic acid at different concentrations (0, 0.2, 0.3, 0.6, 0.7, and  $0.9 \text{ g.L}^{-1}$ ). Analyses were performed in triplicate and polyphenols level was expressed in mg gallic acid equivalents (GAE)/g fresh mass.

Total flavonoids were determined by the method of Marinova et al. [23]. A volume of 0.75 mL of sodium nitrite ( $\text{NaNO}_2$ ) to 5% (w/v) was added to 2.5 mL of extract in a 25 mL flask. The mixture was added 0.75 mL of aluminum chloride ( $\text{AlCl}_3$ ) to 10% (m/v) and incubated for 6 minutes in the dark. After incubation, 5 mL of sodium hydroxide ( $\text{NaOH}$  1N) were added and the volume made up to 25 mL. The mixture was stirred vigorously before being dosed with UV-Visible spectrophotometer. The reading was taken at 510 nm with distilled water as a blank. A calibration curve was performed with quercetin ( $0\text{-}1.5 \text{ g.L}^{-1}$ ) at different concentrations (0, 0.15, 0.3, 0.9, 1.2,  $1.5 \text{ g.L}^{-1}$ ). The tests were performed in triplicate and the flavonoids content was expressed in mg quercetin equivalents (QE)/kg fresh mass.

## **2.7 Extraction and Quantification of Caffeine**

50 mg of ground kola nuts were macerated using ultrasonic cleaner (JP Selecta, S.A) with 10 mL of acidified methanolic solvent (Methanol/acetic acid 1%,  $\frac{1}{4}$  (v/v)) for 30 min. The crude extracts obtained were filtered through a  $0.45 \mu\text{m}$  syringe filter before analytical analyses.

HPLC-DAD analyses were performed according to the method described by Yoe-Ray et al. [24]. The samples ( $20\mu\text{L}$ ) were injected on a reverse phase column XDB-C18,  $4.6\times 150\text{mm}$  (Agilent, USA) with a manual injector (Schimadzu, Japan). Experiments were conducted on a Shimadzu chromatography system equipped with an UV-Visible detector (Shimadzu CR3A). Caffeine was separated at room temperature with a mobile phase of methanol and acetic acid 1% ( $\frac{1}{4}$ , v/v), isocratic elution system. The flow rate was set at  $1.0 \text{ mL.min}^{-1}$  and the detection wavelength was carried out at 274 nm. A total time required for the analysis was 10 min. External standard linear calibration plots were constructed using five points at 0.025, 0.02, 0.015, 0.01,  $0.005 \text{ g.L}^{-1}$ . The chromatograms were identified and confirmed by quantitative results were expressed as mg caffeine equivalents (CAE)/kg fresh mass. All analyses were achieved in triplicate.

The extraction yield was determined from cola extract containing  $2.5 \text{ mg.L}^{-1}$  of standard caffeine. Five separate extractions were performed. The intraday and interday variability at three assay concentrations of caffeine, 5, 10 and  $25 \text{ mg.L}^{-1}$ , were evaluated for five replicates over 5 successive days.

## **2.8 Assessment of Dietary Intake of Polyphenols and Caffeine in Adults Ivorian**

The recommended intake of a nutrient is the average amount of this nutrient supply per person per day to meet the needs of that person and ensure good health. In West Africa, the total amount of fruit consumed daily is 183 g against only 0.6 g of kola nuts [25]. And to calculate nutrient intake of each type of nut, the following equation was used:

$$AN \text{ (mg / kg / day)} = (T \times Q) / P$$

Where AN is the nutrient intake considered; T is the nutrient content (mg/g); Q is the amount of food consumed per day (g) and P the average weight of an adult (60 kg).

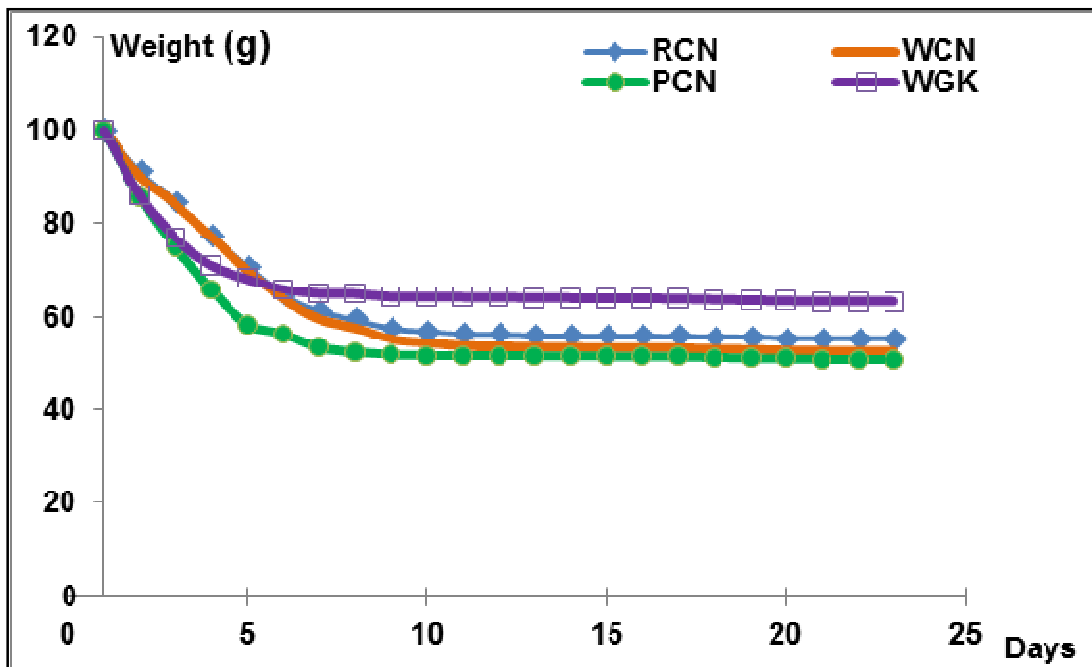
## 2.9 Statistical Analysis

All measurements and analysis were carried out in triplicates. Analysis of variance (ANOVA) was performed to determine the efficiency of the solvent, state and morphotype of nuts as well as establish the differences in content of total polyphenols and flavonoids among cola species. Averages and standard deviations of the analyzed parameters were classified using Newman-Keuls test, significance values was accepted at the  $P = .05$ .

## 3. RESULTS AND DISCUSSION

### 3.1 Kinetic Drying

Fig. 1 shows the drying kinetics of different types of nuts processed. We find that the different curves have the same shape: a decreasing phase of variable duration (first to tenth day of *C. nitida* nuts and the first to the seventh day for *G. kola* nuts) followed by a stationary phase. In addition we observe that *Garcinia kola* species has the lowest content of free water and then follow in descending order, red, white and violet Cola nuts.



**Fig. 1. Kinetics drying of kola nuts**

RCN: Red *C. nitida*; WCN: White *C. nitida*; PCN: Purple *C. nitida*; WGK: White *G. kola*

The falling slope of the drying curves shows that there is a gradual evaporation of free water cells nuts. The slope of these curves varies to one nut to another. That of *G. kola* is lower (4.39) than *C. nitida* (7.41). We can deduce that *C. nitida* nuts drying in this interval is much faster and the percentage of free water in this case is more important. The dry matter content of the species *G. kola* (63.25%) is relatively higher than that of *C. nitida* (from 50.82 to 55.24%). These changes in water evaporation rate observed during drying could be explained by several factors [26] include: a structural difference in the walls of the two species; a significant gap before the drying of moisture and a difference at the contact surface air plant. After this phase, we see a stabilization resulting masses nuts and free water is no longer available. This is usually achieved from the 10th day of drying.

### 3.2 Phytochemical Screening

The phytochemical constituents in kola nuts have been reported to several biological activities [27,1,5]. Phytochemical results of different kola nuts extracts presents in Table 2 indicate that all extract contained flavonoids, alkaloids and saponins, but tannins were only detected in *C. nitida* extracts.

The chemical composition of plants depends on genetic factors, range of the species studied, maturity of fruits and environmental conditions. Phytochemical screening of extracts of *C. nitida* showed the presence of metabolites such as alkaloids, flavonoids and tannins. These results are in agreement with the findings of Reid et al. [28] and Sonibare et al. [11], studies in which they revealed the presence of these metabolites in *C. nitida* nuts. Caffeine is the major alkaloid of *C. nitida* seed [2,29]. Its pharmacological effect includes a stimulation of the central nervous system (CNS) and heart, a coronary vasodilatation, an inhibition of the smooth muscle, stimulation of the skeletal muscles, a diuretic effect as low as metabolic changes that can lead to hypokaliemia and dysglycemia [30]. As for the polyphenolic compounds (flavonoids and tannins), according to literature, their properties are anti-carcinogenic, anti-ulcerous, anti-inflammatory, analgesics, vasodilators, antiviral etc [31]. Thus, the consumption of kola nuts, stimulates the nervous system, suppresses appetite and fight against the physical and mental fatigue, fight against diseases associated with oxidative stress properties, and have aphrodisiac effect due to the presence of caffeine and polyphenols [1,29,8]. In *G. kola*, tests reveal a presence of flavonoids and other molecules that are in trace amounts or absent, results in agreement with those of [20].

**Table 2. Phytochemical screening of kola nut extract**

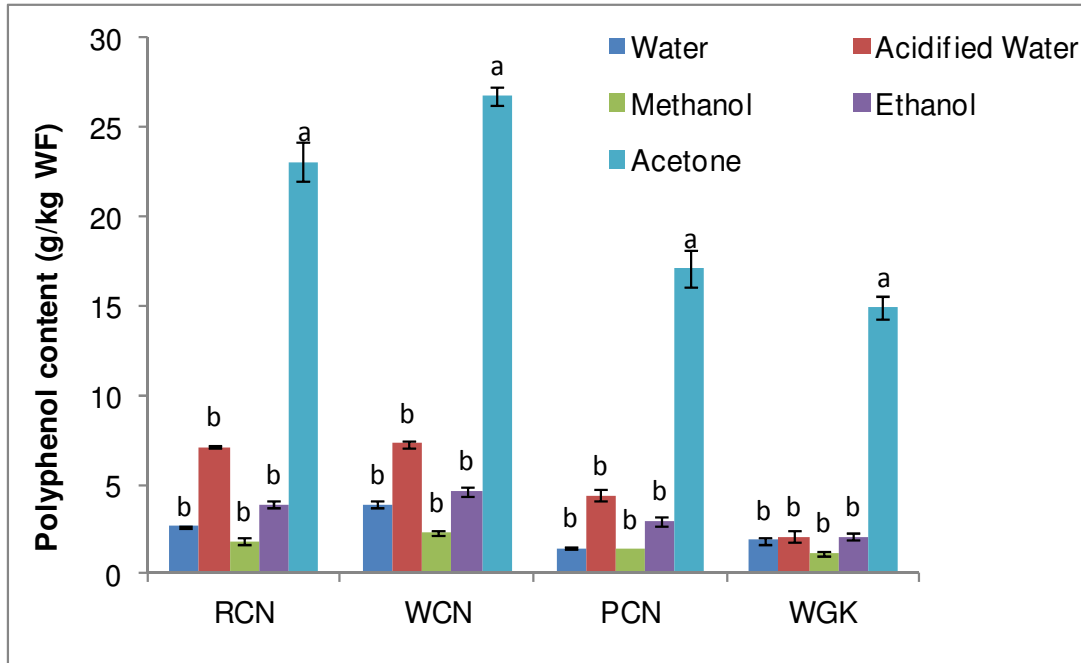
Constituents	RCN	WCN	PCN	WGK
Flavonoids	++	++	++	++
Alkaloids	++	++	++	Traces
Tannins	+	+	+	-
Saponins	++	++	++	++

++: strong presence, +: average presence, -: absence

### 3.3 Total Phenolics Content

Fig. 2 shows the distribution of total polyphenols of different extracts of cola. We find that the most important levels are obtained with nuts *C. nitida*. The magnitude is: WCN > RCN > PCN > WGK regardless the extraction solvent used. The highest rates are obtained by extracting with solvent acetone 60%. The rates are  $26.76 \pm 0.54$ ,  $23.08 \pm 1.12$ ,  $17.06 \pm 0.98$  and

14.90±0.64 mg/g FW for White *C. nitida*, red *C. nitida*, purple *C. nitida* and *G. kola* respectively. Statistical analysis at  $P = .05$ , reveals a significant effect of the solvent type ( $P = 0.000000$ ) used for the extraction of polyphenols.



**Fig. 2. Polyphenolic contents of kola nut extract**  
 data bearing the same letter are not significantly different at  $P = .05$

Polyphenolic contents of kola nuts vary from one species to another. Based on the work of Prohp et al. [15] reported that total polyphenol contents in the *C. nitida* species are proportional to the nuts morphotype. More they are colorful, more important is the extractable. These studies have shown rates of  $33.50 \pm 2.51$ ,  $29.50 \pm 2.67$  mg/g FW for nuts red and white *C. nitida* respectively. Also, Niemenak et al. [1] reported mean levels of total polyphenols at  $57.0 \pm 10.0$  mg/g FW and  $139 \pm 33$  mg/g FW for the *C. nitida* and *G. kola* species respectively. This reflects a high content of total polyphenols in *G. kola* nuts compared to that of *C. nitida* when fresh. These levels are relatively higher than those obtained in this study (14-26 mg/g FW for acetone extracts, and lower for the other extracts). This can be explained by the variability of levels of extractable from one area to another [1], but also and especially by non optimizing conventional mining methods used in our experiments. Indeed, the establishment of better extraction conditions that take into account changes in factors such as the "temperature", the "ratio (w/v)," the "mode" exhaust and "solvent" extraction favor obtaining better rates extractable.

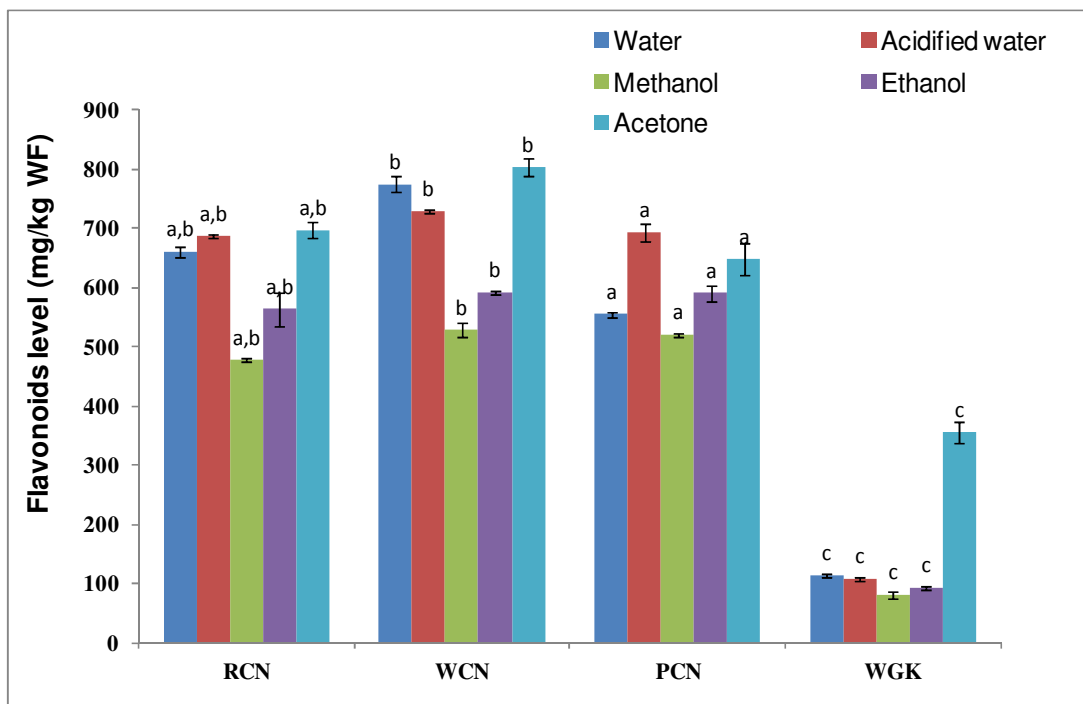
We see from our results that the order of magnitude is perfectly reversed when nuts are dry. The order is as follows:  $WCN > RCN > PCN > WGK$ . According Prohp et al. [15], the content of polyphenol oxidase (responsible for the oxidation of polyphenols enzyme) is higher in the white nuts (WCN) over red nuts (RCN) and purple (PCN). This implies that the activity of this enzyme is higher in WCN, where it's low content in the fresh state. Water loss during drying



would imply stopping oxidation by modifying the optimal activity of enzymes responsible for the degradation and thus the concentration of various metabolites conditions.

### 3.4 Total Flavonoids Content

Fig. 3 shows the distribution of total flavonoids content in different extracts of kola nut studied. Just as the level of total polyphenols, *C. nitida* nuts contain the highest levels of flavonoids compared to *G. kola*, whatever the extraction solvent used. The acetone extracts have the highest flavonoid levels with 803.03±14.48, 697.13±12.76, 647.76±26.33 and 355.75±17.03 mg/kg FW for WCN, RCN, PCN and WGK respectively. Statistical analyzes revealed a significant influence of the factor "morphotype" ( $P = 0.000000$ ) on flavonoids extraction.



**Fig. 3. Total flavonoids content of kola nut extract**  
 data bearing the same letter are not significantly different at  $P = .05$

The contents of total flavonoids in *C. nitida* nuts are greater than those of *G. kola*. These results are in agreement with the work of Hostettmann et al. [32] and Niemenak et al. [1]. Indeed, flavonoids are the major component of *C. nitida* and are found in small amounts in nuts *G. kola*.

### 3.5 Caffeine Content

The caffeine content varies from one species to another. Cola species presents the highest rate with 10812.5±6.27, 10678.4±12.04, 7129.7±3.06, 80.08±2.91 mg/kg FW for red *C. nitida*, white *C. nitida*, purple *C. nitida* and *G. kola* respectively. The highest levels are

obtained with red nuts (RCN). In general, we note that the caffeine content of *G. kola* nuts is very low Table 3.

The presence of high levels of caffeine in *C. nitida* species compared to that of *G. kola* justify the interest of these nuts for Europeans and American industries [29]. Kola nuts are employed in the preparation of various products such as laxative, sedative and non-alcoholic beverages, in particular Coca-Cola. Coca leaves were removed from the original formulation of Coca-Cola in 1904. This soft drink thus no longer contains cocaine but it does have non-negligible quantities of caffeine which originate from extracts of kola nuts [31].

According to the work of Niemenak et al. [1], levels of caffeine are  $13761 \pm 2728$  mg/kg WF in *C. nitida* and absence in extracts of *G. kola*. Based on our work, levels of caffeine in *C. nitida* nuts are significantly lower than those obtained in the work Niemenak et al. [1]. This is due to the lower efficiency of the solvent methanol/1% acetic acid (1/4, v/v) used for the extraction of caffeine in our different samples. As for *G. kola* nuts, we observed greater concentrations. This could be attributed to the drying performed at baseline. In fact, caffeine is largely complexed by the abundance of tannins and catechins when the nuts are fresh (complex formation caffeine and tannins, catechins, caffeine). During drying operation, the oxidative degradation and polymerization of low molecular weight polyphenols induce stabilizing material and therefore the release of caffeine [29].

**Table 3. Caffeine content in kola nut samples**

Samples	Concentration (mg/kg WF)
RCN	$10812.48 \pm 6.27^a$
WCN	$10678.38 \pm 2.03^a$
PCN	$7129.66 \pm 3.06^a$
WGK	$80.08 \pm 2.91^b$

*data bearing the same letter are not significantly different at P = .05*

The regression equation and its correlation coefficient using a linear regression curve from standards solutions was calculated as  $y = 9.10^{07}x + 16803$  and 0.9969, respectively. The concentrations were calculated by peak areas. Detection limits were found at concentrations of  $1.11 \text{ mg.L}^{-1}$  (LOD) and  $1.8 \text{ mg.L}^{-1}$  (LOQ).

The precision of the present chromatographic method was evaluated by measuring the reproducibility, the repeatability and the accuracy was determined by recovery test. The linearity test showed normal distribution. The values of reproducibility for this method on the basis on peak-area ratio for five replicate injections were 0.93-1.93%, 0.12-1.8% for repeatability and the recovery study of caffeine standard solution ranged from 85.5 to 88.8%. The results reflect an accuracy and stability of the chromatography method used.

### 3.6 Dietary intake of Polyphenols and Caffeine

Table 4 shows the contribution of polyphenols and caffeine in different extracts of cola. We note an average intake of 3.99 mg of polyphenols/day or 0.39% of the recommended maximum intake and 1217.13 mg of polyphenols/day or 101.43% for an equivalent daily consumption of 0.6 g (amount of cola consumed per day in West Africa) and 183 g (amount of fruit consumed per day) of kola nut. *C. nitida* nuts have higher intakes of polyphenols to those of *G. kola*. The lowest contributions are 2.66 and 812.72 mg/day (WGK), while the highest are 5.40 and 1646.92 mg/day (WCN) an increase of 49.35%.

As for caffeine, we note for a daily intake of 0.6 g of cola, an average intake of 4.31 mg/day or 1.08% of the recommended intake, and an estimated 1313.05 mg/day intake or 328.6% of the recommended daily intake when considering cola consumption equivalent to that of fruits, or 183 g. The minimum contribution and the maximum caffeine intake are 0.05 mg/day (*G. kola* nut) and 14.66 mg/day (red *C. nitida* nuts).

Kola nuts polyphenols contributions vary from one species to another. Recently, the estimation of the consumption of polyphenols using the database Phenol Explorer showed that the average intake of polyphenols could be estimated at a value between 1 and 1.2 g/day [16] and the caffeine between 200 and 400 mg/day according to Health Canada [17]. We see from our results that the estimated intakes of polyphenols and caffeine to a daily intake of 0.6 g of cola nuts (or 1/20 of a walnut (n =12 g, n =30)) are well below the reference values. This is due to the low consumption of cola nuts by the population. By cons, if kola nuts are considered as one fruit consumed in West Africa (183g daily consumption, 305 times higher than the previous consumption) estimated intakes of polyphenols (1.218 g/day) and caffeine (1.3 g/day) are relatively higher than the reference values (polyphenols: 1-1.2 g/day, caffeine 200-400 mg/day). This implies that the greater the amount of nuts consumed, the greater is the important nutrient. We note that caffeine consumption can create adverse effects due to the introduced amount [30]. They largely vary from one person to another and can be related to the phenomenon of tolerance [17]. Clinical symptoms are anxiety, irritability, insomnia, nausea, muscular vomiting, palpitations and cramps. We note tremors, a psychomotor agitation, arterial hypertension, a tachycardia and a coronary dilatation [24]. The toxicity of caffeine generally occurs with serum concentrations exceeding 25 mg/l, but the correlation between the clinical concentration and effects is weak [30].

**Table 4. Estimated dietary intake in adult Ivorian**

Parameters	Polyphenols				Average
	RCN	WCN	PCN	WGK	
Average (mg/g WF)	7.71	9.00	5.45	4.44	
Estimated average intake (mg/day)	4.62	5.40	3.27	2.66	3.99
Estimated maximum intake (mg/day)	1410.61	1646.92	998.25	812.72	1217.13
	Caffeine				
Average (mg/g WF)	10.81	10.68	7.13	0.08	
Estimated average intake (mg/day)	6.49	6.41	4.28	0.05	4.31
Estimated maximum intake (mg/day)	1978.68	1954.14	1304.73	14.66	1313.05

#### 4. CONCLUSION

Kola nut is a raw material of economic importance for both rural households for some governments in West and Central Africa. Moreover, this study allowed us to reveal that the kola nut contains substantially large proportion of active compounds such as polyphenols (flavonoids in particular) and caffeine who participate in the fight against specific diseases associated with oxidative stress and able to satisfy nutrient needs provided that the daily consumption is much more important. Kola nuts are thus more or less significant source of antioxidant for human health. This work has therefore highlight the possibility of extraction polyphenols (flavonoids) contained in kola nuts with environmentally non-hazardous solvents

(water, acidified water), thereby providing a gain significant extracts, which in through this rich in flavonoids may be assumed potentially bioactive. Unfortunately the amount of cola consumed per day in West Africa is not significant, hence the recommendation of sensitization of all stakeholders in the direction of a higher consumption of kola nuts.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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