



*canephora*. The aim of this work was to extract and quantify caffeine content from two varieties of *Cola nitida* nuts by using UV spectrophotometric method. The effect of extraction solvent and solid-liquid ratio was also tested.

## 2. Material and Methods

### 2.1 Plant Material

A fresh kola nut was used as plant material. They were collected from October 2013 to March 2014 in Lagoon and Bas-Sassandra regions, Cote d'Ivoire. As shown in table 1, collected plant materials were organized and sent to laboratory. They were washed with distilled water, cut into smaller pieces and dried at room temperature (30±2°C) during two weeks. The dried sample was milled into powder using an electric blender, and stored in polybags prior to analysis.

**Table 1:** Sampling of kola nuts

| Sample identification | Fresh nuts color | Variety     |
|-----------------------|------------------|-------------|
| RCN <sub>1</sub>      | Red              | Traditional |
| WCN <sub>1</sub>      | White            | Traditional |
| RCN <sub>2</sub>      | Red              | Improve     |
| WCN <sub>2</sub>      | White            | Improve     |
| PCN                   | Purple           | Traditional |
| PiCN                  | Pink             | Improve     |

RCN: Red *Cola nitida*, WCN: White *Cola nitida*, PCN: Purple *Cola nitida*, PiCN, Pink *Cola nitida*

### 2.2 Chemicals and Standard

All reagent and solvents were analytical grade. Ethanol, methanol, hydrochloric acid and sulfuric acid were purchased from Carlo Erba (Spain), lead (II) acetate trihydrate from Sharlau (Spain). Caffeine was obtained from Sigma Aldrich (USA). Water was purified by a Milli-Q water purification system.

### 2.3 Caffeine Extraction

#### 2.3.1 Effect of extraction solvent

The influence of the extraction solvent was performed based on the work of Xi [21] with slight modifications. The extracts of kola nuts powders were prepared using different solvents (water, methanol 100%, ethanol 100%, methanol 50% in water, ethanol 50% in water and water/ethanol/methanol (WEM) (2/1/1, v/v/v). 5 g of sample was mixed with 100 mL of solvent in the absence of bright light, with shaking (150 trs/min) at laboratory room temperature (22±2°C). After 20 h, the extracts were filtered through a filter paper (Whatman N°1) and stored at 4°C in refrigerator for subsequent determination.

#### 2.3.2 Effect of Solid/Liquid ratio (w/v)

A study was conducted to determine the best solid/liquid ratio (w/v) for better extraction of kola nuts caffeine. By using the best solvent of caffeine extraction as determined in first step, samples were extracted at different solid/liquid ratio, which were 1/100; 3/100; 4/100; 5/100; 6/100 (w/v). Caffeine content for each sample was determined and the best solid/liquid ratio was selected according to the values of five responses.

### 2.4 Determination of Caffeine Content

Caffeine content of kola nuts was measured with spectrophotometric analysis [21]. 20 mL of kola nut extracts with 10 mL of hydrochloric acid (0.01 mol/L) and 2 mL of lead acetate basic solution (50 g Pb(CH<sub>3</sub>COO)<sub>2</sub> Pb(OH)<sub>2</sub> were mixed in 100 mL water and then were collected to stand at least for 12 hours) were mixed with 218 mL water in a 250 mL volumetric flask. At 1 h, the mixture was filtered (solution A). 50 mL of solution A and 0.2 mL of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) at 4.5 mol/L were mixed with 49.8 mL distilled water in a 100 mL volumetric flask. The mixed solution was allowed to stand for 30 minutes and then filtered (solution B). The absorbance of solution B was measured against prepared reagent blank at 274 nm using a 10 mm quartz cell. A good linear calibration for concentrations ranging from 0 to 0.025 mg/mL caffeine was found. The concentration of caffeine determined from the calibration was used to calculate the extraction efficiency of kola caffeine according to equation (1).

The extraction yields of kola caffeine (%)

$$T = \frac{C * V * \left(\frac{250}{20}\right) * \left(\frac{100}{50}\right) * 100\%}{M} \quad (1)$$

Where C was concentration of caffeine in mg/mL; V the total volume of extraction solution in mL; and M the mass of kola powder in mg.

### 2.5 Statistical Analysis

All experiments were done in triplicate and data in tables and figures represent mean values ± standard deviation (n = 3). The statistical analyses were performed by MS Excel 2007 software. Comparison of mean values of measured parameters was performed by a one-way ANOVA (STATISTICA, version 7.1) using LSD test, for the level of significance P=0.05.

## 3. Results and Discussion

### 3.1 Calibration Curve

The absorbance values of seven stocks solutions of standard caffeine were measured. The standard linear calibration curve obtained was shown in Figure 1. We notice clearly a good linear relationship between the absorbance and concentrations of the standard solutions (R<sup>2</sup>=0.997). This statistical measure shows that the regression line approximates the real data points.

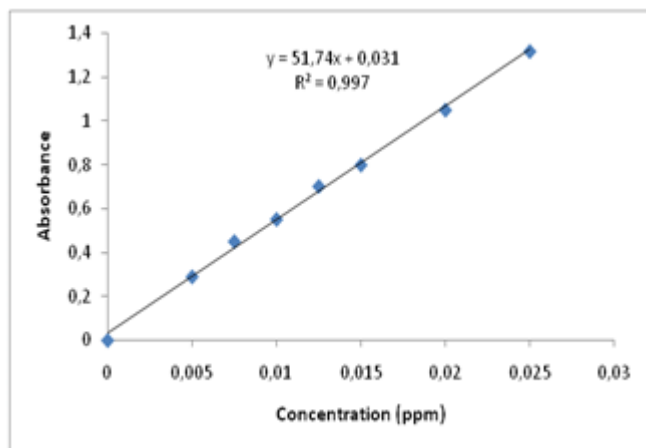


Figure 1: Calibration curve for standard caffeine

### 3.2 Effect of Solvent on Caffeine Extraction

Six solvents were used to extract caffeine from kola nuts: water (100%), methanol (100%, 50% in water), ethanol (100%, 50% in water) and water/ethanol/methanol (WEM) (2/1/1, v/v/v). Caffeine content varies from one solvent to another and content obtained from kola nuts ranged between 0.87-1.95%. The higher and lower amounts of extracted caffeine were obtained with WEM [WEM: Water/Ethanol/Methanol (2/1/1 ; v/v/v) solvent] and ethanol 100% solvent, respectively (Figure 2).

According to Wu *et al* [22] and Mussatto *et al*. [23] the traditional methods of solvent extraction of plant materials are mostly based on the choice of solvents and its concentration, the solid/liquid ratio, time of contact and use of heat and/or agitation to increase the solubility of materials and the rate of mass transfer. The results are in correlation with the findings of Xi [21], where caffeine solubility and rate of mass transfer vary depended on the polarity of the solvent and the organic proportion used.

Statistical analysis revealed a significant difference of caffeine levels obtained from different extraction solvents at  $P=0.05$ . The decreasing order of efficiency extraction solvents is  $WEM^f > Ethanol 50\%^d > Methanol 100\%^a > Methanol 50\%^b > Water^e > Ethanol 100\%^c$ , for the samples  $RCN_1$ ,  $PCN$ ,  $PiCN$ ,  $WCN_2$  and  $WEM^f > Ethanol 50\%^d > Methanol 100\%^a > Methanol 50\%^b > Water^e > Ethanol 100\%^c$  for the samples  $RCN_2$  and  $WCN_1$ . We note that whatever the sample use, the difference efficiency orders of solvent is at the amount obtained starting from methanolic extracts (Methanol 100% and Methanol 50%)

Caffeine content increase as we move from solvents water, ethanol, methanol and their mixture with water (50% in water) to WEM solvent. These increases are 5.84%, 13.87%, 14.17%, 77.92% and 92.96% for ethanol 50%, methanol 50%, methanol 100%, water and ethanol 100%, respectively.

In general, we note that concerning the same morphotype, caffeine content of improved variety is greater than that of traditional variety ( $RCN_2 > RCN_1$  and  $WCN_2 > WCN_1$ ). This observation was probably due to the greater tendency of improved variety for storing alkaloids/caffeine due to genetic

differences [13]. According to Odebo [24] there are intra-species differences in *C. nitida*. Also, in some regions genetically modified kola are planted by the National Center of agricultural research (CNRA) to increase the yield and reduce astringency [4], thus it affects the phytochemical composition. Several reports indicate that external stimuli can modulate the synthesis and change composition or quantities of phytochemicals in plants. Generally, the level of phytochemicals in crops is affected by several factors, such as genetic differences (variety or cultivar), environmental conditions, crop management practices (e.g. nutrient availability, application of insecticides, fungicide and herbicides) [13],[3].

The general magnitude of content is  $WCN_2 > PiCN > RCN_2 > PCN > WCN_1 > RCN_1$  (Figure 2).

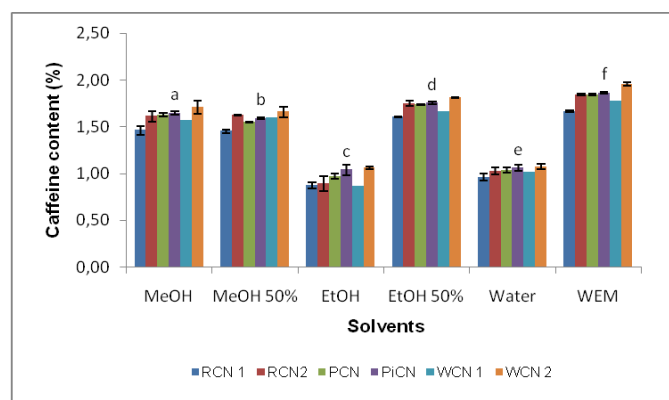


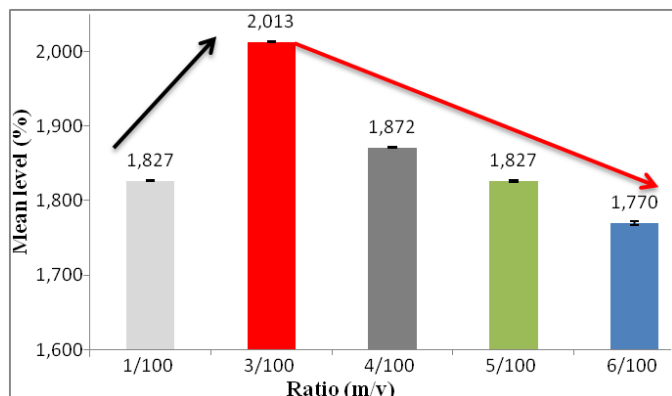
Figure 2: Effect of different solvents on the extraction yields of kola nuts caffeine.

*RCN*: Red *Cola nitida*, *WCN*: White *Cola nitida*, *PCN*: Purple *Cola nitida*, *PiCN*, Pink *Cola nitida*  
 1: traditional variety 2: improved variety

### 3.3 Effect of Solid/Liquid Ratio (w/v)

Figure 3 shows that regardless of the sample studied, caffeine content increased generally when solid/liquid ratio increased from 1/100 to 3/100 (g/mL), and then decreases until ratio 6/100. According to Xi [21], dissolution of bioactive components into the solvent is a physical process. We note in the first part (ratio ranging between 1/100 to 3/100), when the amount of kola nuts powder increases, the chance of bioactive components coming into contact with the solvent goes up, which leads to higher leaching-out rates. In the other hand, for solid/liquid ratio ranging between 3/100 to 6/100, content decreasing may be basically due to the saturation phenomenon. In fact when the solvent is saturated on bioactive components, the cellular phenomenon of diffusion stops and there has stabilization rate of extracted compounds or decreased.

Caffeine mean values obtained from kola nuts ranged between 1.77 % to 2.01%. The higher and lower amounts of extracted caffeine were obtained with 3/100 and 6/100 solid/liquid ratio, respectively.



**Figure 3:** Effect of Ratio on extraction yields

RCN: Red Cola nitida, WCN: White Cola nitida, PCN: Purple Cola nitida, PiCN: Pink Cola nitida

1: traditional variety 2: improved variety

The solid/liquid ratio 3/100 was the optimum ratio for kola nuts caffeine extraction. On average, there is an increase in content of 10.18% (1.827-2.013) and a decrease of 12.07% (2.013-1.770). Specifically, increase are 6.86%, 6.59%, 1.57%, 5.46%, 2.22% and 38.38% for RCN<sub>1</sub>, RCN<sub>2</sub>, PCN, PiCN, WCN<sub>1</sub> and WCN<sub>2</sub> respectively when ratio increased from 1/100 to 3/100 and, the decrease of 14.43%, 7.73%, 7.22%, 5.69%, 6.52% and 26.56% for RCN<sub>1</sub>, RCN<sub>2</sub>, PCN, PiCN, WCN<sub>1</sub> and WCN<sub>2</sub> respectively when ratio increased from 3/100 from 6/100 (Table 2).

The difference observed generally in caffeine content is related to sample amount used and the solubility of caffeine in the proportion of the solvent available. The lowest amount of caffeine for this solid/liquid ratio (3/100) was observed in WCN<sub>1</sub> sample (1.84%), while WCN<sub>2</sub> sample showed the highest (2.56%) (Table 2). This content is comparable with that obtained in *Cola nitida* by other workers of 2.42% and 1.5-3.8% respectively [19],[1], but higher than content obtained by Atanda *et al.* [5]. Variation depending on the variety of nut characterized, as well as the treatment of kola nut and the extraction procedure. The highest caffeine content from this study is within the range that will not be detrimental to health. Excessive consumption of kola nut without medical prescription can have some adverse effect on the health of the consumer [1]. According to Rawlings and Thompson [25], concentration of 100g/kg must be exceeded by any consumer for it to have serious adverse effect on health.

Statistical analysis shows that whatever the ratio used, the lowest content was obtained with the sample RCN<sub>1</sub> and higher with WCN<sub>2</sub>. As previously experience, contents of improved nuts are higher than that of traditional nuts.

| Ratio (m/v)      | 1/100      | 3/100      | 4/100       | 5/100      | 6/100      |
|------------------|------------|------------|-------------|------------|------------|
| RCN <sub>1</sub> | 1.75±0.05* | 1.87±0.02* | 1.71±0.01*  | 1.67±0.01* | 1.60±0.02* |
| RCN <sub>2</sub> | 1.82±0.03* | 1.94±0.02* | 1.89±0.02** | 1.84±0.08* | 1.79±0.01* |
| PCN              | 1.91±0.03* | 1.94±0.02* | 1.87±0.01*  | 1.85±0.01* | 1.80±0.03* |
| PiCN             | 1.83±0.05* | 1.93±0.04* | 1.89±0.01** | 1.86±0.01* | 1.82±0.01* |
| WCN <sub>1</sub> | 1.80±0.03* | 1.84±0.01* | 1.83±0.01*  | 1.79±0.01* | 1.72±0.05* |
| WCN <sub>2</sub> | 1.85±0.02* | 2.56±0.02* | 2.00±0.02*  | 1.95±0.02* | 1.89±0.06* |
| Average          | 1.827±0.04 | 2.013±0.18 | 1.872±0.06  | 1.827±0.07 | 1.770±0.07 |

Data of the same column having the same sign are statistically different by the LSD test at P=0.05

RCN: Red Cola nitida, WCN: White Cola nitida, PCN: Purple Cola nitida, PiCN: Pink Cola nitida

1: traditional variety 2: improved variety

#### 4. Conclusion

Results obtained in this study showed that *C. nitida* are rich source of caffeine and therefore can be used to produce new added value products. This richest justify energy drinks and pharmaceuticals industries interest for this plant material for the preparation of various products such as laxative, sedative and non-alcoholic beverages, in particular Coca-cola and Pepsi-cola.

In our research, UV/VIS spectrophotometric method employed for the quantification of caffeine in kola nuts was found to be relatively easy, fast and cheap. It does not require expensive solvents and reagents, it may be recommended for the rapid, precise and sensitive quantification of caffeine in kola nuts. The optimum conditions found for caffeine extraction were: solvent Water/Ethanol/Methanol (2/1/1, v/v/v) and solid-liquid ratio 3/100 (m/v). However, methanol is a toxic solvent, and ethanol is non-toxic, it easy to recycle and mixes with water in different ratios. So taking into account the cost, a moderate concentration of ethanol solution (50% ethanol in water) must be chosen as solvent to optimize caffeine extraction from kola nuts in the following studies.

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