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Proximate and Phytochemical of *Cola nitida* and *Cola acuminata*

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Abstract: The aim of the research was to examine *Cola nitida* and *Cola acuminata* for their phytochemical and proximate compositions. Presence of secondary metabolites do provide information about the plants for their potentials as a lead candidates for the novel drug discovery. The proximate analysis was done using the method of Association of Official Analytical Chemists (AOAC) and the phytochemical analysis was done using methods of Markkar and Goodchild for tannin, Brunner for saponin, Harbone for alkaloid and Bohm and Koupai-Abyazani for flavonoid. The proximate results showed that the moisture content of *Cola acuminata* and *Cola nitida* were in the range of 9.73-9.81%, ash 2.72-2.21%, fat 3.02-2.20%, protein 19.14-15.24%, crude fiber 7.30-4.18% and carbohydrate 58.09 66.45%. *Cola acuminate* has more protein content, ash and fat than *Cola nitida*. The result of phytochemical analysis showed that *Cola acuminata* has more alkaloids (2.22%), tannin (6.46%) and saponin (8.06%) than *Cola nitida*. The phenol contents of the two kola nuts were the same range 0.27%, the flavonoid were in the range of 0.12-0.14%. The presence of secondary metabolites in these plants are indications that if well researched, novel bioactive compounds can be discovered in them as there are worldwide efforts by scientists looking for new bioactive compounds to combat various ailments which have developed high resistant to already known antibiotics.

Key words: *Cola nitida*, *Cola acuminata*, proximate and phytochemical compositions

INTRODUCTION

Cola nitida and *Cola acuminata* are among various species of cola, they are eating by elderly people. The greatest concentration of *Cola nitida* is in the forest area of Ivory Coast and Ghana, *Cola acuminata* is in the area stretching from Nigeria to Gabon. *Cola nitida* is the kola which has social and traditional significance. Kola contains about two percent caffeine and is chewed by many people as a stimulant. It is used in the manufacture of dyes (Javies, 2002), it is also used in the manufacture of the cola group beverages- coca-cola, Pepsi cola and kola (Javies, 2002). Plants have been used since antiquity for medicinal purposes by diverse peoples and cultures throughout the world. Indeed, the recorded use of natural products as a source of relief from illness dates back at least four thousand years and it can be assumed that unrecorded practices are as old as mankind (Christophersen *et al*., 1991). The use of plants for medicinal purposes continues to this day, usually in the form of traditional medicine, which is now recognized by the World Health Organization (WHO, 2005) as a building block for primary health care (Akerele, 1988; WHO, 2005). The vibrant healing power of herbs had been recognized since creation and hence botanical medicine is one of the oldest practiced professions by mankind (Van Wyk and Gericke, 2000; Iwu, 1993). Hamburger and Hostettmann (1991) reported that 25% of prescribed drugs today are of plants origin. Well-known examples of drugs with plant origins includes aspirin, atropine, digoxin, ephedrine, morphine, quinine, reserpine, vincristine and vinblastine, as well as several plant steroidal2sapogenins which serve as semi-synthetic precursors to the steroidal drugs. The study of plants of medicinal importance in the first years of the nineteenth century led to the isolation in crystalline form of such complex substances as Strychnine ($C_{21}H_{22}O_2N_2$), Quinine ($C_{20}H_{24}O_2N_2$) and Morphine ($C_{17}H_{19}O_3N$) which have physiological actions in man and animals (Farnsworth and Bingel, 1997). Herbal medicine has for too long been neglected in favor of synthetic drugs of which its misuse or abuse and cases of side effects have become a social evil (Farnsworth, 1990).

However, the essence of the research is to examine the proximate and phytochemical of *Cola nitida* and *Cola acuminata*.

MATERIALS AND METHODS

The kola nuts were obtained from king's market in Akure on the 4th of July, 2012 and the samples identity were confirmed by crops, soil and pest department, Federal University of Technology, Akure, Nigeria. The

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kola nuts were cured by the traditional method of wrapping in fresh banana leaves to reduce the amount of moisture lost and later sun dried for five days, it was grinded into powdered form using blender.

Proximate analysis: Standard methods of the Association of Official Analytical Chemists (AOAC, 1984) were used to determine the moisture, crude protein, crude fat, total ash and crude fibre contents of each sample. Moisture content was determined by heating three gram of each sample to a constant weight in a crucible placed in an oven maintained at 105°C. The dry matter was used in the determination of the other parameters. Crude protein (%total nitrogen×6.25) was determined by the Kjeldahl method, using three gram samples; crude fat was obtained by exhaustively extracting five gram of each sample in a Soxhlet apparatus using petroleum ether (boiling point range 40-60°C) as the extractant. Ash was determined by the incineration of seven grams samples placed in a muffle furnace maintained at 550°C for 5 h. Crude fibre was obtained by digesting four grams of sample with H_2SO_4 and NaOH and incinerating the residue in a muffle furnace maintained at 550°C for 5 h. Total carbohydrate was obtained by difference. Each analysis was carried out in triplicate and Anova statistical method was used.

Phytochemical analysis

Tannin determination: Finely grounded sample was weighed (0.2 g) into a 50 mL sample bottle. Ten of 70% aqueous acetone was added and properly covered. The bottle was put in an ice bath shaker and shaken for 2 h at 30°C. The solution was then centrifuge and the supernatant stored in ice, 0.2 mL of the solution was pipette into the test tube and 0.8 mL of distilled water was added. Standard tannin acid solution was prepared from a 0.5 mg mL^{-1} of the stock and the solution made up to 1 mL with distilled water, 0.5 mL of Folinciocateau reagent was added to the sample and standard followed by 2.5 mL of 20% Na_2CO_3 the solution was then vortexed and allow to incubate for 40 min at room temperature, its absorbance was read at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid curve prepared (Markkar and Goodchild, 1996).

Saponin determination: The spectrophotometric method of Brunner (1984) was used. Two gram of the finely grinded sample was weighed into a 250 mL beaker and 100 mL of Isobutyl alcohol was added. Shaker was used to shake the mixture for 5 h to ensure uniform mixing. The mixture was filtered using No. 1 Whatman filter paper into 100 mL beaker containing 20 mL of 40% saturated solution of magnesium carbonate. The mixture obtained again was filtered using No. 1 Whatman filter paper to obtain a clean colourless solution. One milliliter was added into 50 mL volumetric flask using pipette, 2 mL of 5% iron (iii) chloride ($FeCl_3$) solution was added and made up to the mark with distill water. It was allowed to stand for 30 min for the colour to develop. The absorbance was read against the blank at 380 nm:

$$\text{Saponin} = \frac{\text{Absorbance of sample} \times \text{Conc. of standard}}{\text{Absorbance of standard}} \quad (1)$$

Alkaloid determination: Five gram of the sample was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and allowed to stand for 4 min, this was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was alkaloid which was dried and weighed (Harbone, 1973):

$$\%\text{Alkaloid} = \frac{W_3 - W_2}{W_1} \times 100\% \quad (2)$$

Where:
W_1 = Initial weight of sample
W_2 = Weight of the extract
W_3 = Final weight of the residue

Flavonoid determination: Ten gram of the sample was extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered using Whatman filter paper No. 42 (125 mm). The filtrate was transferred into crucible and evaporated into dryness over water bath and weighed to a constant weight (Bohm and Koupai-Abyazani, 1994).

Determination of phenol: The fat free sample was boiled with 50 mL of ether for the extraction of the phenolic component for fifteen min. Five mL of the extract was pipette into a 50 mL flask and then 10 mL of distilled water was added. Two milliliter of ammonium hydroxide solution and 5 mL of amyl alcohol were also added to the sample and made up to the mark. It was left to react for 30 min for colour development; the absorbance was measured at 550 nm.

RESULTS AND DISCUSSION

The result of proximate composition (Table 1) shows that the moisture content of the two samples were

Table 1: Result of the proximate analysis of the kola nut species

Parameters (%)	*Cola acuminata*	*Cola nitida*
Moisture	9.73±0.02[a]	9.81±0.01[b]
Ash	2.27±0.01	2.21±0.01[b]
Fat	3.02±0.01[a]	2.20±0.01[a]
Protein	19.14±0.25	15.24±0.58[a]
Crude fiber	7.30±0.25[b]	4.18±0.09[a]
Carbohydrate	58.09±0.89	66.45±0.53

All the mean for the group are similar according to DMRT, ($p<0.05$) Duncan's multiple ranges test

Table 2: Result of the phytochemical analysis of the kola nut species

Parameters (%)	*Cola acuminata*	*Cola nitida*
Alkaloid	2.22±0.01[b]	0.22±0.02[a]
Phenol	0.27±0.00[b]	0.26±0.00[b]
Tannin	6.46±0.01[c]	5.92±0.01[b]
Flavonoid	0.12±0.01[a]	0.14±0.01[a]
Saponin	7.30±0.25[b]	1.07±0.07[a]

All the mean for the group are similar according to DMRT, ($p<0.05$) Duncan's multiple ranges test

similar, *Cola acuminate* (9.73%±0.02) and *Cola nitida* (9.81%±0.01). This is expected since the samples have been subjected to drying for five days to reduce the moisture content. High moisture content is an index of spoilage, the protein content of *Cola acuminate* (19.14%±0.25) was high than *Cola nitida* (15.24%±0.58), also the crude fiber of *Cola acuminate* (7.30%±0.25) and carbohydrate value (68.09%±0.89) were higher than that of *Cola nitida* of crude fiber (4.18%±0.09) and carbohydrate (66.45%±0.53). The proximate composition of kola nut differs relatively from what has been reported by Arogba (1999) that dried kola nut had 69% carbohydrate, 18% crude fat and 3.1% ash dried weight. The varying composition as reported by various workers may be due to different environment where the plants were being planted, seasons and climatic condition.

The result of phytochemical analysis in percentages (Table 2) shows that *Cola acuminata* has high content of tannin (6.46%±0.01), saponin (8.06%±0.07), alkaloid (2.22%±0.01) than that of *Cola nitida* of tannin content (5.92%±0.01), saponin (1.07%±0.07) and alkaloids (0.22%±0.02). The phenol and flavonoid were very small, *Cola acuminate* having phenol content of (0.27b±0.00), flavonoid (0.12%±0.01) and *Cola nitida* having phenol (0.26b±0.02) and flavonoid (0.14a±0.01). The phytochemical test of *Geranium incanum* showed that alkaloid was absent, but tannin and flavonoids were present in different concentrations (Bruneton, 1999; Amabeoku, 2009).

CONCLUSION

From the results of proximate analysis, it is quite interesting that *Cola acuminata* has more protein content and crude fibre than *Cola nitida* and also the presence of high content of tannin, saponin and alkaloid in *Cola acuminata* is an indication that if further research can be done on the sample, novel bioactive compounds can be derived from it after isolating the compounds and characterizing them using various spectroscopic techniques.

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