



## A Comparative Study of the Phytochemical Activities of Some Nigerian Indigenous Kola Nuts *Kola acuminata* (Igbo Kola Nut), *Kola vera* (Hausa Kola Nut), and *Garcinia kola* (Bitter Kola)

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### Authors' contributions

This work was carried out in collaboration among all authors. Authors SEI and MCE designed the study, authors SEI and EOA performed the statistical analysis, authors SEI and MCE wrote the protocol and author MCE wrote the first draft of the manuscript. Authors MCE, SEI, EOA, ULK, NEN, ICC and CGO managed the analyses of the study. Authors SEI and MCE managed the literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** Nigerian indigenous kola nuts (*Garcinia kola*, *Kola acuminata*, *Kola vera*) were evaluated for potential phytochemical properties.

**Study Design:** Phytochemical analysis.

**Place and duration of Study:** Renaissance University, Ugbawka, Enugu State, Nigeria, July 2016.

**Methodology:** The nuts were dried, ground and extracted by cold maceration with 99.5% methanol for 72 hours after which the methanol was allowed to evaporate.

**Results:** The phytochemical evaluation revealed the presence of saponin glycosides, glycoside, volatile oil, steroid and alkaloid in *Kola vera*; saponin, saponin glycoside, glycoside, tannins,

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pseudo tannins, volatile oil, steroid and alkaloid in Kola acuminata while flavonoid, alkaloid and steroid were found in *Garcinia kola*.

**Conclusion:** The phytochemical activities results showed that kola acuminata and *Garcinia kola* extracts exhibited more phytochemical than *kola vera*.

**Keywords:** Phytochemical; Nigeria; indigenous kola nuts; kola acuminata; kola vera; *Garcinia kola*.

## 1. INTRODUCTION

Nigeria being of one of the countries in African continent, medicinal plants are the main sources of drugs for traditional and modern medical practice, food supplements and pharmaceuticals. They have been utilized in the treatment of human diseases for many years because they contain important organic compounds that can produce definite physiological actions both in human bodies and animals, known as plant phytochemicals [1] They contain the tannins, flavonoids terpenoids, saponins, phenolic compounds and alkaloids with excellent therapeutic activities and usually low or no toxicity [2]. Considerable number of African diseases are treatable with medicinal plants especially kola nuts [3]. Phytochemicals and antioxidants found in kola nuts have been successfully used in the management of oxidative stress related diseases [4,5]. Alkaloids found in kola nuts are effective in the reduction of fever and headache [6]. Kola nuts as important medicinal plants like some essential oils are used in combating certain fungal strains [7] and generally, Nigerian kola nuts are used to treat bacterial infections [8]. In spite of the fact that most major Nigerian kola nuts have considerable phytochemical and antioxidant activities and therefore protect against diseases, only a few researches have been reported on the enormous health benefits of these kola nuts [9]. Moreover, most of the previous researches have been on single kola nuts not on comparative basis. Considering the enormous health benefits of these medicinal plants, we were inspired to evaluate the comparative phytochemical activities of some Nigerian indigenous kola nuts, so as to contribute to reduction and elimination of health related problems that are preventable and curable by consumption of kola nuts with phytochemicals and antioxidants activities.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Reagents

Preparation of 0.5 M solution of sodium hydroxide using 1000 cm<sup>3</sup> volumetric flask.

Molar mass of NaOH = 23+16+1 = 40 g/mol

Mass of NaOH required =  $0.5 \text{ mol} \times 40 \text{ g} = 20 \text{ g/dm}^3 \text{ dm}^3$

20 g of NaOH is for 1000 cm<sup>3</sup>.

**Procedure:** 20 g of NaOH was weighed and dissolved in 1000 cm<sup>3</sup> volumetric flask and was made up to the mark with distilled water.

Preparation of 0.030 M ferric chloride solution using 1000cm<sup>3</sup> volumetric flask.

Molar mass of FeCl<sub>3</sub>=35.5x3+56 = 162.5 g/mol

Molar mass of FeCl<sub>3</sub> required =  $0.30 \text{ mol} \times 162.5 \text{ g} = 48.75 \text{ g/dm}^3 \text{ dm}^3$

48.75 g/dm<sup>3</sup> of FeCl<sub>3</sub> is for 1000 cm<sup>3</sup>,

**Procedure:** 48.75 g of FeCl<sub>3</sub> was dissolved in 1000 cm<sup>3</sup> volumetric flask and made up to the mark with distilled water.

Preparation of 0.1 mol/dm<sup>3</sup> tannic acid using 100cm<sup>3</sup> volumetric flask.

Molar mass of Tannic Acid (C<sub>76</sub>H<sub>52</sub>O<sub>46</sub>) = 12x76+1x52+16x46= 1704 g/mol

Mass of C<sub>76</sub>H<sub>52</sub>O<sub>46</sub> required =  $0.1 \text{ mol} \times 1704 \text{ g} = 17.04 \text{ g/dm}^3$

17.04 g/dm<sup>3</sup> of C<sub>76</sub>H<sub>52</sub>O<sub>46</sub> is for 1000 cm<sup>3</sup>

**Procedure:** 17.040 g of C<sub>76</sub>H<sub>52</sub>O<sub>46</sub> was introduced to the 1000 cm<sup>3</sup> volumetric flask and made up to the mark with distilled water.

Preparation of 1Moldm<sup>-3</sup> solution of Ammonia acid using 100 cm<sup>3</sup> volumetric flask.

1 Moldm<sup>3</sup> solution of Ammonia contain 99g/dm<sup>3</sup>.

Therefore 1 moldm<sup>3</sup> solution of ammonia acid would contain 99 gdm<sup>3</sup>.

Since the acid stock solution contain 98% of ammonia acid with relative density of 0.88. Mass =  $98/100 \times 0.88 = 0.87 \text{ g}$ .

0.87 g of ammonia is contained on 1cm<sup>3</sup> of stock solution 98 of the acid will contain.

$$\frac{98}{0.87} \times 1 \text{ cm}^3 = 11.32 \text{ cm}^3$$

The volume of the concentrated ammonia acid required to be diluted to give 1.00moldm<sup>3</sup> solution =11.32 cm<sup>3</sup>.

Preparation of 0.1 Moldm<sup>3</sup> solution of Ethanol Acid using 100 cm<sup>3</sup> Volumetric flask.

0.1 Moldm<sup>3</sup> solution of Ethanol acid contain 99 g/dm<sup>3</sup>.

Then 0.1 Moldm<sup>3</sup> solution of ethanol acid would contain 99 gdm<sup>3</sup>.

If the acid stock solution contain 98% of ethanol acid with relative density of 0.78.

Then Mass = 98/100 x 0.78= 0.75  
0.75 g of ethanol acid is contained on 1 cm<sup>3</sup> of stock solution 98 of the acid will contain.

$$\frac{98}{0.75} \times 1 \text{ cm}^3 = 13.1 \text{ cm}^3$$

Therefore volume of the concentrated ethanol acid needed to be diluted to give

1.00 moldm<sup>3</sup> solution =13.1 cm<sup>3</sup>.

## 2.2 Sample Collection and Preparation

In this research work, three types of kola nut were used, they are *kola Vera* (Hausa kola), *kola acuminata* (Igbo kola), and *kola garcinia* (bitter Kola). The kola nuts were collected from Eke-Agbani Market, in Nkanu west LGA, Ori-agu market in Udi LGA of Enugu State, and Ogbete Main Market, respectively all in Enugu State, South-Eastern of Nigeria.

### 2.2.1 Sample preparation

The samples were washed and sliced into tiny pieces and allowed to dry for 98 hours under room temperature condition. The dried samples were crushed into fine powder to increase the surface area activities.

### 2.2.2 Extraction using cold maceration methods

200 g each of the three ground kola samples, *kola acuminata*, *kola vera* and *Garcinia kola* was weighed and poured into three different beakers with firm covers. 99.5% methanol was employed

for the extraction. The first stage of the extraction was done using 1000ml of ethanol to soak each samples for a period of 24 hours with intermittent stirring, after which the extract was filtered and squeezed with teflon cloth to remove all the liquid and a second filtration was done with filter paper (Whatman No 20 ), then the kola nuts materials were recovered, the extracts were then transferred into an empty storage bottles and labelled appropriately. Then, the kola nuts materials (residues) were reintroduced into the three different buckets for second extraction. Similarly, the second extraction was carried out using 500 ml of 90% methanol to soak each of *Garcinia kola*, *kola acuminata* and *kola vera* for 24 hours with regular stirring. The kola nuts material recovered and the extract were poured into the storage bottles according to its label. The same procedure was repeated for the third time using 250 ml ethanol and the extracts were poured into the labelled bottles; the extraction lasted for 72 hours. Finally, the extracts were evaporated in an open air to obtain the crude extracts which were weighed and recorded, the percentage yields were also calculated and recorded.

## 2.3 Phytochemical Analysis

### 2.3.1 Test for saponins [10]

2.5 cm<sup>3</sup> of each extract was vigorously shaken with 10 cm<sup>3</sup> of water for 2 minutes in a test tube. 2cm<sup>3</sup> of olive oil was then added. It was observed for persistent frothing and emulsion formation and result recorded.

### 2.3.2 Test for saponin glycosides [10]

2.5 cm<sup>3</sup> of mixture of Fehling's solutions A and B were added to 2.5 cm<sup>3</sup> of each extract in a test tube. Development of bluish-green precipitate was of interest here and the result was recorded.

### 2.3.3 Test for steroids and triterpenoids (Libermann-Burchard test)

2 cm<sup>3</sup> of acetic anhydride were added to 2 cm<sup>3</sup> of each extract in a test tube and was cooled in ice. 3 cm<sup>3</sup> of concentrated Sulphuric acid was carefully added and a change in colour from violet to bluish-green colour was observed.

### 2.3.4 Test for glycoside (General) [10]

Dilute Sulphuric acid (2.5 cm<sup>3</sup>) were added to 5 cm<sup>3</sup> of each extract in a test tube and boiled for

15 minutes. Then 3 cm<sup>3</sup> of 0.5 mol/dm<sup>3</sup> sodium hydroxide and 5 cm<sup>3</sup> of mixed Fehlings solution A and B were added. The formation of brick-red precipitate is a positive test.

### 2.3.5 Test for digitalis glycosides [11]

A drop of 0.030mol of ferric chloride were added to 2 cm<sup>3</sup> of each extract in a test tube; 2 cm<sup>3</sup> of glacial acetic acid and 2 cm<sup>3</sup> of each concentrated sulphuric acid were added. The resulting solution was observed for the formation of blue layer and the result was recorded.

### 2.3.6 Test for anthracenes (Born-Tragger's test)

2 cm<sup>3</sup> of chloroform was added to 2 cm<sup>3</sup> of each extract in a test tube and was allowed to separate. To the chloroform layer were added 2 cm<sup>3</sup> of 1 mol/dm<sup>3</sup> of ammonia solution and vigorously shaken and kept to separate. The observation of brick-red precipitate is a positive result and was recorded.

### 2.3.7 Test for tannins (general) [11]

A mixture of 4 cm<sup>3</sup> of each extract and 4 cm<sup>3</sup> of water in a test tube was stirred very well and 3 drops of 0.30 mol/dm<sup>3</sup> ferric chloride solution added and the mixture observed for immediate green colouration and result recorded. (Trease and Evan, 1978).

### 2.3.8 Hydrolisable tannins [10]

4 cm<sup>3</sup> of 1 mol/dm<sup>3</sup> of Ammonia solution were added to 4 cm<sup>3</sup> of each extract in a test tube and shaken very well and observed for the formation of an emulsion and the result was recorded.

### 2.3.9 Test for pseudotannins [12]

A matchstick was dropped into 3 cm<sup>3</sup> of each extract in a test tube and 2 drops of concentrated hydrochloric acid added. The matchstick was then left undistorted for 5 minutes and observe for a dark purple colouration on it and result was recorded.

### 2.3.10 Test for flavonoids [11]

A small quantity of magnesium chips was dropped in 3 cm<sup>3</sup> of each extract in a test tube and 5 drops of concentrated hydrochloric acid

added. The formation of reddish colouration is a positive result and it was recorded.

### 2.3.11 Test for resins [11]

2 cm<sup>3</sup> of acetic anhydride were added to 2 cm<sup>3</sup> of each extract in a test tube and 2 drops of concentrated sulphuric acid added. It was observed for violet colouration and the result was recorded.

### 2.3.12 Test for alkaloids

**(a)Kranz Test:** Two drops of Kranz reagent were added to 2 cm<sup>3</sup> of each extract in a test tube and observed frosty milky solutions.

**(b)Tannic Acid Test:** Two drops of 0.1 mol/dm<sup>3</sup> (W/V) tannic acid were added to 2 cm<sup>3</sup> of each extract in a test tube and observed for a cream colouration and the observation was recorded.

**(c) Mayer's Test:** Three drops of Mayer's reagent were added to 2 cm<sup>3</sup> of each extract in a test tube and this was observed for a reddish precipitation or colouration.

### 2.3.13 Volatile oil test [11]

Six drops of 0.30 mol/dm<sup>3</sup> ferric chloride solution were added to a mixture of 2 cm<sup>3</sup> of each extract in a test tube and 2 cm<sup>3</sup> of 0.1 mol/dm<sup>3</sup> (V/V) ethanol. The resulting mixture was observed for green colouration and the result was recorded.

## 3. RESULTS

### 3.1 Result of Crude Extract and Percentage Yield

Kola nuts	Crude extract
<i>Kola vera</i>	54.60 g
<i>Garcinia kola</i>	45.20 g
<i>Kola acuminata</i>	36.60 g
<b>Percentage yield</b>	
% yield	= $\frac{\text{weight of crude extract}}{\text{initial weight of sample}} \times 100$
<b><i>Kola vera:</i></b>	
$(54.60 / 200) \times 100 = 27.3\%$	
<b><i>Garcinia kola:</i></b>	
$(45.20/200) \times 100 = 22.6\%$	
<b><i>Kola acuminata:</i></b>	
$(36.60/ 200 ) \times 100 = 18.3\%$	

**Table 1. Phytochemical results**

	<b>Phytochemical</b>	<b><i>Kola vera</i></b>	<b><i>Kola acuminata</i></b>	<b><i>Garcinia kola</i></b>
1	Saponin	-	+	-
2	Saponin Glycoside	+	+	-
3	Glycoside	-	+	-
4	Digitalis Glycoside	-	-	-
5	Anthracene	+	-	-
6	Tannins	-	+	-
7	Hydrolysable tannins	-	+	-
8	Pseudo tannins	-	+	-
9	Flavonoids	-	+	+
10	Resin	-	-	-
11	Volatile oil	+	+	-
12	Alkaloid			
12a	Tannic acid	-	-	-
12b	Krants test	+	+	+
13	Steroid	+	+	+

Key: + = Present, - = Absent

Results of Phytochemical screening of the crude extract of the three varieties of kola nuts (*kola vera*, *kola acuminata*, *Garcinia kola*) is shown in Table 1.

#### 4. DISCUSSION

From the results, *Vera kola* had the highest percentage yield (27.3% crude extract) amongst the three kola nuts used in this research, seconded by *Garcinia kola* (22.6%) and then *kola acuminata* has the smallest percentage yield (18.3%).

The phytochemical and antioxidant study carried out on the three varieties of kola nuts, (*Garcinia kola*, *kola acuminata* and *kola vera*) revealed the presence of medicinally active constituents. The phytochemically active compounds of the three varieties of kola were qualitatively analyzed and the results presented in Table 1. Saponin glycosides, glycoside, volatile oil, steroid and alkaloid were found in *kola vera*; saponin, saponin Glycoside, Glycoside, Tannins, Pseudo tannins, Volatile oil, Steroid and Alkaloid were found in *kola acuminata* while flavonoid, Alkaloid and steroid were found in *Garcinia kola*. The medicinal values of the kola nuts lie in their constituent phytochemicals and antioxidants [13]. From the chemical analysis it was observed that all the three samples of kola nuts contained phytochemicals, out of the three samples *kola acuminata* (Igbo kola nut) has the most phytochemical properties followed by *kola garcinia* (bitter kola) and then *kola vera* (Hausa kola nut). Also all of them contain antioxidants properties. The phytochemicals and antioxidants

play a vital role in delaying, intercepting, and preventing oxidation reaction catalyzed by free radical in the body thereby preventing related sicknesses.

#### 5. CONCLUSION

The present study revealed that Nigerian indigenous kola nuts contained high amount of phytochemicals and antioxidants. The observed phytochemical and antioxidant activities of these kola nuts justified their medicinal use for prevention and cure of diseases. The presence of the identified phytochemicals makes the kola nuts pharmacologically active. Their antioxidant activity may be responsible for their usefulness in the management and treatment of various diseases. *Kola acuminata* is the most potent among the three varieties of Nigerian kola nuts in prevention of cancer and other health related issues since antioxidants help in eliminating the carcinogenic radicals in human body. We also recommend that *Kola acuminata* (lbo kola nut) should be consumed more than *kola vera* (Hausa kola nut) and *Garcinia kola* (bitter kola) because of it high phytochemical and antioxidants content.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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