Original Research Article

A Comparative Study of the Phytochemical Activities of Some Nigerian Indigenous Kola Nuts *Kola Acuminate* (Igbo kola nut*), Kola Vera (*Hausa kola nut*), and Garcinia kola (*Bitter kola)

ABSTRACT

Aims: Nigerian indigenous kola nuts (Garcinia Kola, Kola acuminate, Kola vera) were evaluated for potential phytochemical properties. **Study design:** Phytochemical analysis. **Place and Duration of Study:** Renaissance University, Ugbawka, Enugu State, Nigeria, 2016. **Methodology:** The nuts were dried, ground and extracted by cold maceration with 90% 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, pseudo tannins, volatile oil, steroid and alkaloid in kola acuminate while flavonoid, alkaloid and steroid were found in Garcinia kola. **Conclusion:**The phytochemical activities results showed that kola acuminate and garcinia kola extracts exhibited more phytochemical than kola vera.

Keywords: Phytochemical, Nigeria, Indigenous Kolanuts, *Kola acuminate, 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]. 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 research have been reported on the enormous health benefits of these kola nuts[3]. Moreover, most of the previous research have been on single kolanuts 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. MATERIAL AND METHODS

2.1Standardization of reagents

Preparation of 0.5M solution of sodium hydroxide using 1000cm^3 volumetric flask.

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

Mass of NaOH required = 0.5mol x 40g= 20g/dm^3 dm^3

20g of NaOH is for 1000cm^3..

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

Preparation of 0.030M ferric chloride solution using1000cm^3 volumetric flask.

Molar mass of $FeCl_3$=35.5x3+56 = 162.5g/mol

Molar mass of $FeCl_3$ required = 0.30mol x 162.5g= 48.75g/dm^3 dm^3

48.75g/dm^3 of $FeCl_3$ is for 1000cm^3,

Procedure: 48.75g of $FeCl_3$ was dissolved in 1000cm^3 volumetric flask and made up to the mark with distilled water.

Preparation of 0.1mol/dm^3 tannic acid using 100cm^3 volumetric flask.

Molar mass of Tannic Acid ($C_{76}H_{52}O_{46}$) =12x76+1x52+16x46=

1704g/mol

Mass of $C_{76}H_{52}O_{46}$ required = 0.1mol x 1704g= 17.04g/dm^3

17.04g/dm^{3}of $C_{76}H_{52}O_{46}$ is for 1000cm^3

Procedure: 17.040g of $C_{76}H_{52}O_{46}$ was introduced to the 1000cm^3 volumetric flask and made up to the mark with distilled water.

Preparation of 1Moldm-^{3} solution of Ammonia acid using 100cm^3 volumetric flask.

1Moldm^3 solution of Ammonia contain 99g/dm^3.

Therefore 1moldm^3 solution of ammonia acid would contain 99gdm^3

Since the acid stock solution contain 98% of ammonia acid with relative density of 0.88. Mass = 98/100 x0.88=0.87g

0.87g of ammonia is contained on 1cm^{3}of stock solution 98 of the acid will contain

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

The volume of the concentrated ammonia acid required to be diluted to give 1.00moldm^3 solution =11.32cm^3

Preparation of 0.1Moldm^{3}solution of Ethanol Acid using 100cm^{3}Volumetric flask.

0.1Moldm^3 solution of Ethanol acid contain 99g/dm^3.

Then 0.1Moldm^3 solution of ethanol acid would contain 99gdm^3

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

0.75g of ethanol acid is contained on 1cm^3 of stock solution 98 of the acid will contain

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

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

1.00moldm^3 solution =13.1cm^3.

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-AgbaniMarket,in Nkanu west LGA, Orie-agu market in Udi LGA of Enugu State, and Ogbete Main Market, respectively all in Enugu State, South-Eastern of Nigeria

2.2.1Sample 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.

2.2.3 Extraction using Cold Maceration Method

200g 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. 90% 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 500ml 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 250ml 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[4]

2.5cm^3 of each extract was vigorously shaken with 10cm^3 of water for 2 minutes in a test tube. 2cm^3 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 [4]

2.5cm^3 of mixture of Fehling's solutions A and B were added to 2.5cm^3 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 $cm^3$of acetic anhydride were added to 2cm^3 of each extract in a test tube and was cooled in ice. 3cm^3 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) [4]

Dilute Sulphuric acid (2.5cm^3) were added to 5cm^3 of each extract in a test tube and boiled for 15 minutes. Then 3cm^3 of 0.5mol/dm^3 sodium hydroxide and 5cm^3 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[5]

A drop of 0.030mol of ferric chloride were added to 2cm^3 of each extract in a test tube; 2cm^3 of glacial acetic acid and 2$cm^3$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)

2cm^3 of chloroform was added to 2cm^3 of each extract in a test tube and was allowed to separate. To the chloroform layer were added 2cm^3 of 1mol/dm^3 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) [5]

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

2.3.8 Hydrolisable Tannins [4]

4cm^3 of 1mol/dm^3 of Ammonia solution were added to 4cm^3 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 [6]

A matchstick was dropped into 3cm^3 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 [5]

A small quantity of magnesium chips was dropped in 3cm^3 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[5]

2cm^3 of acetic anhydride were added to 2cm^3 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)Krants Test: Two drops of Krants reagent were added to 2cm^3 of each extract in a test tube and observed frosty milky solutions.

(b)Tannic Acid Test : Two drops of 0.1mol/dm^3 (W/V) tannic acid were added to 2cm^3 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 2cm^3 of each extract in a test tube and this was observed for a reddish precipitation or colouration.

2.3.13 Volatile Oil Test [5]

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

3. RESULTS AND DISCUSSION

3.1 Result of Crude Extract and Percentage Yield

Kola nuts	Crude extract
Kola vera	54.60g
Kola Garcinia	45.20g
Kola Acuminata	36.60g

Percentage Yield

$$\% \text{ yield} = \frac{\text{weight of crude extract}}{\text{initial weight of sample}} \times 100$$

Kola vera:

54.60 X 100 = 27.3%

Kola Garcinia:
45.20 X 100 = 22.6%
200

KolaAcuminata:
36.60 X 100 = = 18.3%
200

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

Table 1: Phytochemical results

	Phytochemical	***Kola vera***	***Kola acuminata***	***Kola Garcinia***
1	Saponin	-	+	-
2	Saponin Glycoside	+	+	-
3	Glycoside	-	+	-
4	Digitalis Glycoside	-	-	-
5	Anthrancene	+	-	-
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

3.2. 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 *kola Garcinia*(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 acuminate* 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 Table1.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 acuminate* while flavonoid, Alkaloid and steroid were found in *Garcinia kola*. The medicinal values of the kola nuts lie in their constituent phytochemicals and antioxidants[7]. From the chemical analysis it was observed that all the three samples of kola nuts contained phytochemicals, out of the three samples *kola acuminate*(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.

4. 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* (Ibo kola nut) should be consumed more than *kola vera*(Hausa kola nut) and *kola garcinia* (bitter kola) because of it high phytochemical and antioxidants content.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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