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# Phytochemical and antioxidant properties of the Nigeria Kola: *Kola acuminata* (Oji Igbo) *Kola vera* (Oji Hausa) and *Garcinia kola* (Bitter Kola)

**Ali Bilar and Ibekwe Prisca Akudo**

**Abstract**
Qualitative and quantitative phytochemical and antioxidant of analysis of *Kola acuminata, Kola vera* and *Garcinia kola* was carried out to determine the presence of alkaloids, tannins, flavonoids, saponins and glycosides. The result revealed that *Garcinia kola* (bitter kola) has high content of flavonoid (9.70± 0.30) and saponin (9.23±0.55) followed by *Kola vera* (6.93±0.42) and (6.30±0.36) and least in kola acuminate (6.00±0.20) and (4.40±0.50). *Kola vera* has the highest content of tannins (4.44±0.2) and glycosides (2.31±0.17) compared with *Garcinia kola* and kola acuminate with tannin and glycode content as (3.56±0.04) and (1.43±0.06) and (2.23±0.04) and (1.57± 0.26) respectively. Kola acuminate has the highest content of alkaloids (2.7±0.12) followed by *Kola vera* and *Garcinia kola*. The result of the antioxidant activities showed that *Garcinia kola* has the highest percentage of the antioxidant activities for nitric oxide scavenging capacity, reducing power, hydrogen peroxide scavenging activity and anthacyanin content followed by kola acuminate and *Garcinia kola*.

**Keywords:** phytochemical, antioxidant, qualitative and quantitative

## Introduction
Kola nut is a plant that grows in a coastal rain forest in western and south eastern part of Nigeria, bitter kola which is known as *Garcinia kola* is an African wonder nut which comes from *Garcinia kola* forest which belong to the family of clusiaceae [1]. *Kola acuminata* and *Kola vera* belong to the family of sterculiaceae and are particularly very common among the Yorubas of south western Nigeria.

*Kola vera* called 'goro' by the Hausa which is more abundant an of commercial variety consumed by ECOWAS region. Kola acuminate is called 'Ori Igbo' Ly the lgbo's which is the variety grown exclusively in lgbo land [2]. Traditionally these nuts are chewed as a masticators substance to stimulate the flow of saliva but are widely consumed as snacks in west and central Africa [1].

Kola nut contain large amount of caffeine, threobromine and kolanin which are the three chemicals that work in synergy as stimulants. The caffeine in the nut also acts as a bronchodilator, expanding the bronchial air passages; hence kola nut is often used to treat whooping cough and asthma [4]. Other medicinal use of kola nut include natural remedy for chest cold which research has prove its efficacy, increases body metabolism, prevents prostate cancer in men and some bacteria infections. Kola nut is one of the best medicines for dysentery, diarrhoea and anorexia (loss of appetite) etc. Kola nut prevents ulcer, stimulate blood circulation and offers potentiating effect to oral analgesics such as aspirin.

Phytochemicals are biologically active naturally occurring compounds found in plants which provide health benefits for humans a medicinal ingredients and nutrients. They protect plants from disease and damage and also contribute to the plants colour, aroma and flavour.

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals thereby leading to chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid (Vitamin C) terminate these chain reactions.

The aim and objectives of this research is to investigate the phytochemical and antioxidant properties of Kola acuminate (Oji Igbo), *Kola vera* (oji Hausa) and *Garcinia kola* (bitter kola).

## Materials and Methods

### Collection of Samples

*Kola vera* and *Garcinia kola* were bought in Ama Hausa Douglas Owerri in Imo state and *Kola acuminata* was bought at Eke Onunwa, Owerri imo State.

### Sample Preparation

The samples was washed and sliced into tiny pieces and allowed to dry for days under room temperature. The dried samples were crushed into fine powder using hand grinder and the fine powder were transferred into plastic bags and properly labelled for further use.

### Sample Extraction Using Cold Maceration Method

100g 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 and labelled respectively.

200m1 of 98% ethanol was used to soak each sample for a period of 24 hours with intermittent stirring. After the 24 hours, the extract was filtered and squeezed with a Teflon cloth to remove all the liquid then the extract was re-filtered using a filter paper. Finally the extract was evaporated in an open air to obtain the crude extract which was poured in to the storage bottle and labelled accordingly.

### Phytochemical Screening

### Test for Alkaloids

The crude ethanoic extract of the dried powdered sample was evaporated to dryness and the residue re-dissolved in about 50ml of 2M HCL and filtered. 5 drops of Meyers reagent was added to the filtrate. A precipitate was formed which indicates the presence of alkaloids.

### Test for Tannins

0.5g of the dried samples was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green colouration.

### Test for Saponins

5mls of the extract solution was placed in a test tube and 5mls of water was added, the mixture was shacked vigorously. The froth formation observed indicated the presence of saponins.

### Test for Flavononoid

2ml of extract was added to concentrated HCI (5 drops) and magnesium ribbon. Pink reddish colour indicates the presence of flavonoids.

### Test for Glycosides

2.5mls of 5% sulphuric acid was added to 5m1 of the extracts in a test tube, the mixture was heated in boiling water for 15 minutes, then cooled and neuiralised with 10% NaOH and 5ml of fehling's solution was added, the mixture was then boiled. A brick red precipitate indicates the presence of glycosides.

### Quantitative Determination of Phytochemicals

### Determination of Alkaloids

10g of the sample was soaked with 80m1 of ethanol and 20ml acetic acid for 4 hours. The mixture was then filtered with a filter paper and the filtrate was heated for 20minutes. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. 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.

### Determination of Tannins

1g of the sample was dispersed in 10ml distilled water and agitated. This was left to stand for 30mins at room temperature being shaken for every 5mins. At the end of the 30minutes, it was centrifuged and 2.5mls of the extract was dispersed into a 50mls volumetric flask. Similarly, 2.5mls of standard tamiic acid solution was dispersed into a separate 50m1 flask, 1ml of Folin Denis reagent was added unto each flask followed by 2.5mls of saturated $Na_2CO$ solution. The mixture was diluted to mark in the flask (50mls) and incubated for 90mins at room temperature. The absorbance was measured at 760nm in UV-Spectrophotometer. Absorbance reading was taken for blank. The tannin content was calculated.

### Determination of Saponins

10g of the plant sample was extracted repeated with 100ml of 80% aqueous ethanol at room temperature. The whole solution was filtered through wattman filter paper. The filtrate was later transferred into a conical flask and evaporated into dryness over a water bath and weighed to a constant weight.

### Determination of Flavonoids

10g of the plant sample was extracted repeated with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through wattman filter paper. The filtrate was later transferred into a conical flask and evaporated into dryness over a water bath and weighed to a constant weight.

### Determination of Glycosides

5g of the sample was weighed and added to 50ml of distilled water in a corked conical flask. The mixture was allowed to stay overnight for proper extraction of the cyanides. After which it was filtered. Different concentrations of HCN containing 2 to 10mg cyanides were prepared. 4ml of alkaline picrate solution (prepared by dissolving 1g of picric acid and 5g of $Na_2CO_3$ in minimal warm in 200ml volumetric flask and made up to 200ml with water) was added to 1ml of the filtrate in a test tube and the mixture was incubated for 30minules for colour development. The absorbance of the samples were taken in a UV-spectrophotometer at 490nm. This was also followed for the different HCN solution. The cyanide content was determined from a calibration curve of the different HCN concentration.

### Determination of antioxidant properties

### Determination of reducing power

1ml of the extracts (concentration 2, 4, 6, 8, and 10mg/ml respectively), 1.0ml of sodium phosphate buffer (0.2M,pH 5.6) and 1.0ml of potassium cyanide (10mg/ml) were mixed and incubated at 50°C for 20mins. Then, 1ml of 10%TCA was added to the mixture and centrifuged for 5mins. 1ml of the supernalant was mixed with 0.1ml of $H_2O$ and 0.1ml of 0.1% ferric chloride and the absorbance was measured at 750nm.

### Determination of Anthocyanin Content

3g of the dried powdered samples were extracted with 10ml of methanolic HCI for 24hours at room temperature in darkness. The solutions were filtered with filler paper and the sample extraction procedure was repeated by adding 4ml of 1% methanolic HCI to the residue and the filtrate was

combined. The combined filtrate was measured at 535nm.

**Determination of Nitric Oxide (No) Scavenging Activity**

An aliquot 6ml of nitroprusside solution was mixed with 6m1 of the extract and incubated at 25 ºC for two and half hour. 0.5ml of the reaction mixture was removed at 30mm intervals and then mixed with 0.5ml of Griess reagents (1% sulphanilamide, 2%phosphoric acid and 0.1% napthylethylene diamine dihydrochioride). The absorbance of the mixture was then read at 546nm and compared with absorbance of 1mg/ml standard solution (sodium nitrate) treated in the same way with Griss reagent.

**Determination of Hydrogen Peroxide Scavenging Capacity**

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffer saline (pH 7.4). Different concentration of plant extract and standard ascorbic acid solution *viz*; 20, 40, 60, 80 and 100ml/g in methanol (1ml), were separately added to $H_2O_2$ solution (2ml), and mixed. Each tube was allowed to stand for 10mins after which absorbance at 230mn was read against a blank solution containing phosphate buffer in place of $H_2O_2$.

**Result and Discussion**

From the qualitative test carried on three Nigeria kola: Kola acuminate, *Kola vera* and *Garcinia kola*; alkanoids, tannins, saponins, flavonoids and glycosides were all present in the three samples as shown in the table 1 below

**Table 1:** Phytochemical screening of kola acuminate, *Kola vera* and *Garcinia kola*

| Parameters | *Kola acuminata* | *Kola vera* | *Garcinia kola* |
|---|---|---|---|
| Alkaloids | + | + | + |
| Tannins | + | + | + |
| Saponins | + | + | + |
| Flavonoids | + | + | + |
| Glycosides | + | + | + |

Key: + = Present = Absent

**Table 2:** Mean value of the phytochemical composition of kola acuminate, *Kola vera* and *Garcinia kola*.

| Parameters | *Kola acuminata* | *Kola vera* | *Garcinia kola* |
|---|---|---|---|
| Alkaloids | 2.75±0.12 | 1.79±003 | 1.84±0.09 |
| Tannins | 2.23±0.04 | 4.44±0.02 | 3.56±0.04 |
| Saponins | 4.40±0.50 | 6.30±0.36 | 9.23±0.55 |
| Flavonoids | 6.00±0.20 | 6.93±0.42 | 9.70±0.30 |
| Glycosides | 1.57±0.26 | 2.31±0.17 | 1.43±0.06 |

Values are mean of three independent determination ± standard deviation.

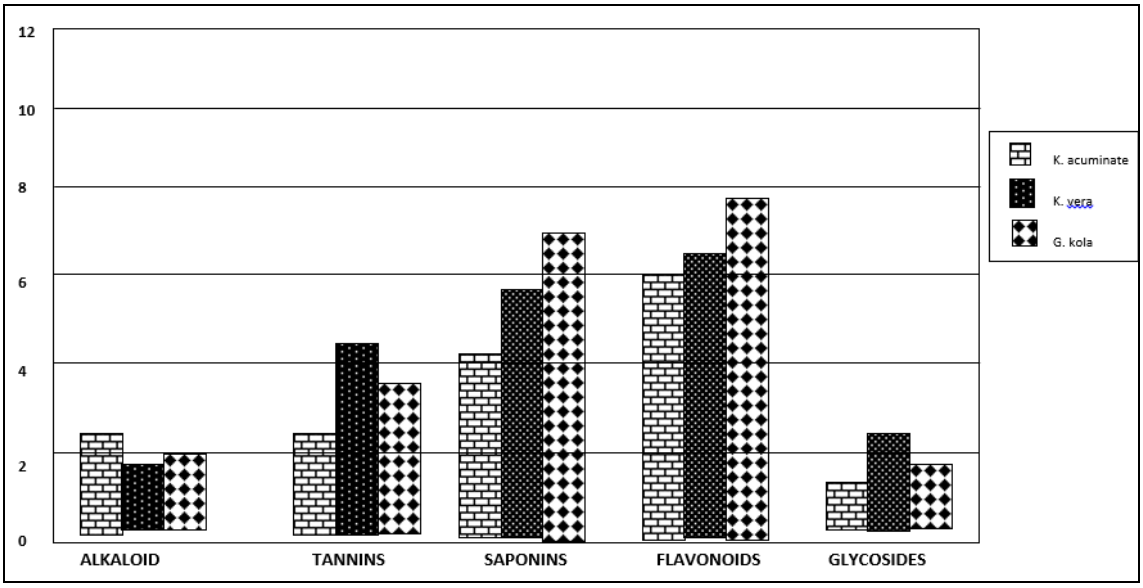


**Fig 1:** Phytochemical composition of kola acuminate, *Kola vera* and *Garcinia kola*.

**Table 3:** Nitric oxide (NO) scavenging activity

| Percentage Activity | | | Time (minutes) |
|---|---|---|---|
| *Kola acuminata* | *Kola vera* | *Garcinia kola* | |
| 30.20 | 23.438 | 61.058 | 30 |
| 32.18 | 25.52 | 61.618 | 60 |
| 33.714 | 27.47 | 68.456 | 90 |
| 34.331 | 33.73 | 70.49 | 120 |
| 38.39 | 34.855 | 74.48 | 150 |

Values are expressed % activity.

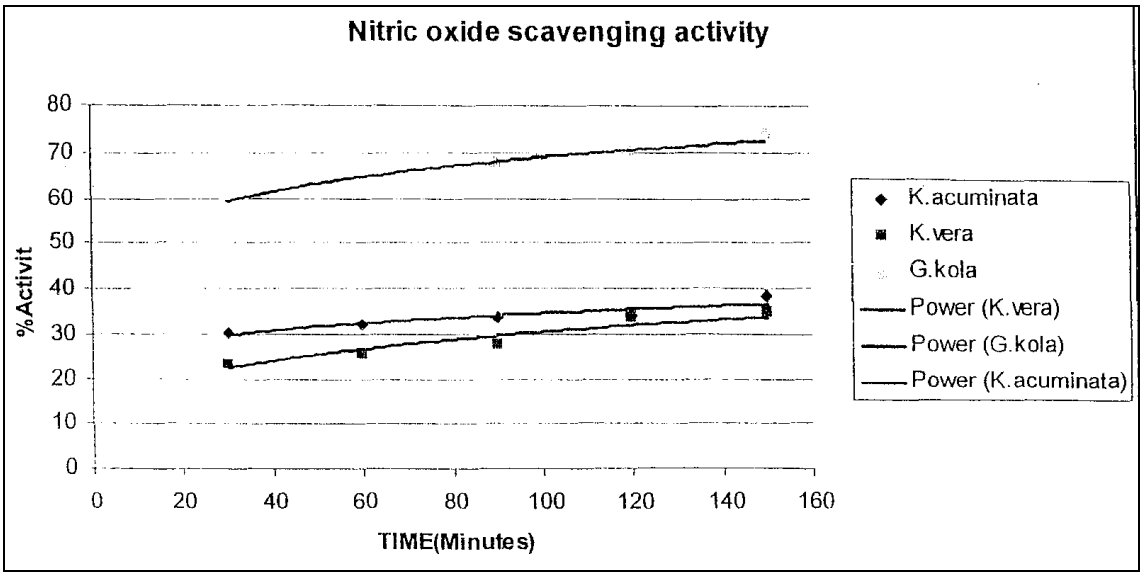


**Fig 2:** Show graph of Nitric oxide scavenging activity against time.

**Table 4:** Hydrogen peroxide scavenging capacity against concentration

| Percentage Activity | | | |
|---|---|---|---|
| ***Kola acuminata*** | **Concentration (mg/ml)** | ***Kola vera*** | ***Garcinia kola*** |
| 65.172 | 20 | 38.339 | 69.990 |
| 54.100 | 40 | 45.695 | 70.43 |
| 48.257 | 60 | 36.597 | 65.58 |
| 48.385 | 80 | 34.188 | 68.45 |
| 54.100 | 100 | 37.160 | 68.89 |

Values are expressed as & activity.

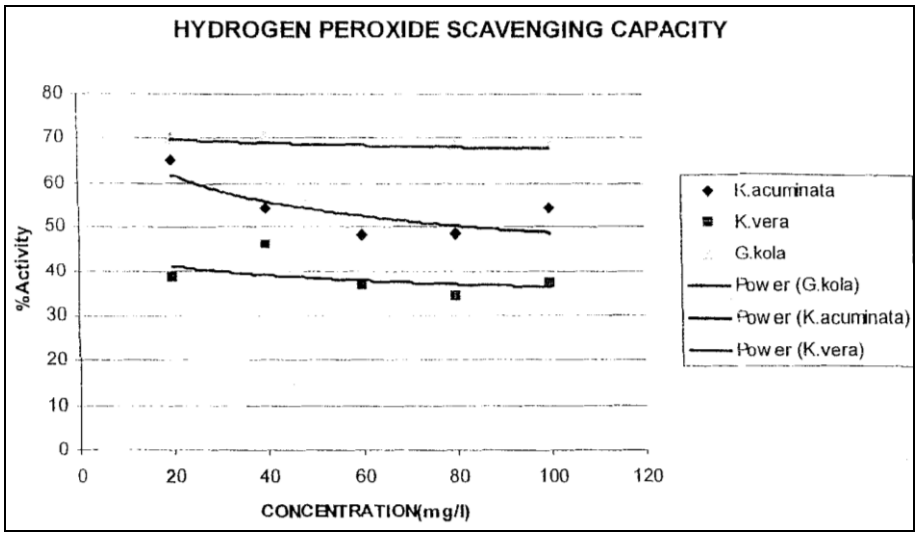


**Fig 3:** Graph showing hydrogen peroxide scavenging capacity against concentration (mg/l)

**Table 5:** means values of the anthancynin content

| Sample | Mean± STDEV |
|---|---|
| Kola acuminate | 4.27±1.123 |
| *Kola vera* | 10.35±0.369 |
| *Garcinia kola* | 2.46±0.977 |

Values are expressed in mean ± standard deviation.

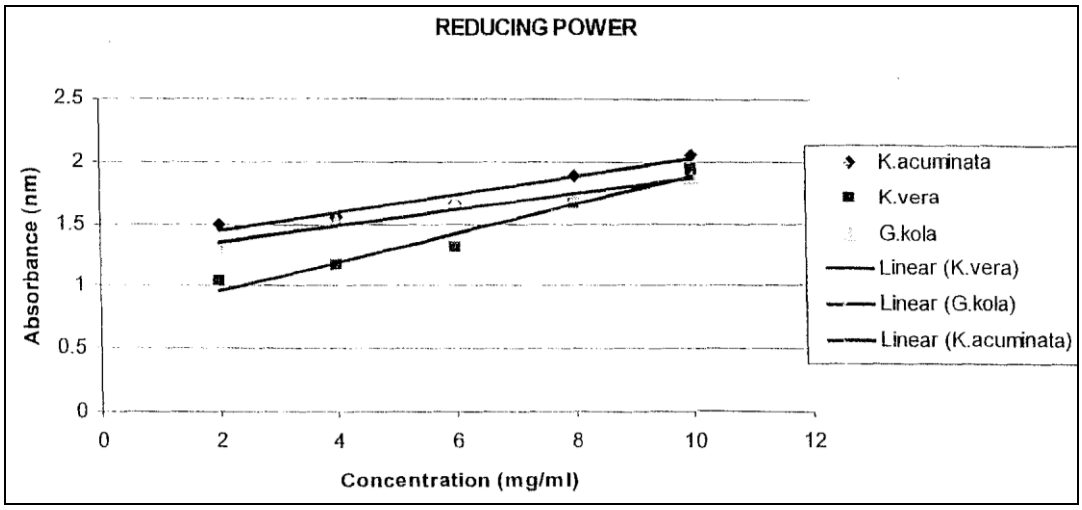


**Fig 4:** Graph showing reducing power against concentration mg/l

## Discussions

Table 1 shows the result of the qualitative analysis carried out on the three kola nut varieties; *Kola acuminata*, *Kola vera* and *Garcinia kola*. The result reveals the presence of alkaloids, tanninis, saponins, flavonoids and glycosides in the three kola nut varieties.

Table 2 and fig 1 reveals the quantitative phytochemical analysis carried out on the three kola nut varieties. From the result, *Garcinia kola* (bitter kola) has the highest content of flavonoids (9.78±0.30) and saponin (9.23±0.55) followed by *Kola vera* (6.93±0.42) and (6.30 ±0.3 6) and least in *Kola acuminata* (6.00±0.20). *Kola vera* has the highest content of tannins (4.44±0.2) and glycosides (2.3 1±0.17) compared with *Garcinia kola* and *Kola acuminata* with tannin and glycoside content as (3.56±0.04) and (1 .43±0.06) and (2.23±0.04) and (1.57±0.26) respectively. *Kola acuminata* has the highest content of alkaloids (2.75±0.12) followed by *Kola vera* and *Garcinia kola*.

From table 3 and fig 2 above, that shows the result of nitric oxide scavenging activity of the three kola nut varieties. It was observed that the nitric oxide scavenging activity increased as the concentration increases. NO shows dual function as both an antioxidant and pre-oxidant depending on the relative ratios of the reactions [8] Antioxidant effects of NO occurs when NO reacts with alkoxy and peroxyl radicals intermediates during lipid peroxidation thereby stabilizing the inhibition of LDL oxidation while the pro-oxidant reaction occurs when NO reacts with 02 to yield peroxynitrite (ONOO) [5]. The ability of Hibiscus sabdariffa to scavenge NO and especially its deleterious metabolite, (ONOO) will be highly beneficial in biological system as ONOO and some other NO metabolites have been implicated in various pathological conditions such as malaria, cardiovascular diseases, inflammation, cancer and diabetes [8].

From table 4 and fig 3 above shows the result of hydrogen peroxide as an active oxygen species has been reported to come mainly from its potential to produce the highly reactive hydroxyl radical through the fenton reaction: $Fe^2 + H_2O_2$ $Fe^3$ + OH + OH [7] Therefore, inhibition of lIP formation will prevent further generation of radicals. Although l-IP on its own is not very reactive, but it could induce ceP death *in vitro* and attack many cellular energy- producing systems *in vivo*. For instance, it has been reported that TIP could deactivate the glycolytic enzyme glyceraldehydes-3 -phosphate dehydrogenase [3, 4, 5].

Fig 4 above shows the result of reducing antioxidant power. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [1]. For the measurements of the reductive ability, it has been found that the Fe3+- fe2+ transformation occurred in the presence of extract samples which were postulated previously by [12]. Earlier authors [9] have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductions [11], which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [6]. Reductions are also reported to react with certain precursors of peroxide, thus preventing peroxide formation.

## Conclusion

The present study revealed that Nigerian indigenous kola nuts; *Kola acuminata*, *Kola vera* and *Garcinia kola* 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. *Garcinia kola* is the most potent among the three varieties of Nigerian kola nuts as it contains high phytochemical and antioxidant content.

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