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# Analysis of Phytochemical Constituents and Antioxidant Potential of Bitter Kola Leaf Extract towards Bioactive Food, Nutrition and Health Resources

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**Abstract**

Plants are important to life, beyond nutritional role there are other functions that plant plays in helping humans and animals survive in their environment. This study focused on identifying the phytochemicals, bioactive compounds present in bitter kola leave, their in-vitro antioxidant activities and compared with n-hexane, ethanol and aqueous solvent to identify effective solvent for extraction of these phytochemicals in relation to food, health and nutritional benefits. The FTIR and GC- MS assessment of the extracts showed the existence of bioactive compounds. The outcome showed the detection of tannin, phenol, alkaloid, flavonoid, protein and carbohydrate in all leaf extracts, with ethanol having the highest concentration of these phytochemicals ($p<0.05$). The in-vitro free radical scavenging properties of bitter kola leaf extracts against 1, 1-diphenyl-2-picryl hydrazyl showed that the n-hexane leaf extract recorded the highest inhibitory activities when compared to both n-hexane and ethanol extracts ($p<0.05$) across all concentrations. However, all extracts showed increase in inhibitory activities with increasing concentrations. When compared to both n-hexane and ethanol leaf extracts, the ferric reducing antioxidant capacity of Garcinia kola leaf extracts rose with concentrations, with the aqueous leaf extract having the strongest reducing characteristics from 5mg/ml to 7mg/ml ($p<0.05$). The bioactive compounds present in bitter kola leaves makes it consumable and suitable in the treatment of different diseases such as bacterial infection, cancer, diabetics and inflammation because its level of effectiveness is confirmed in traditional medicine.

**Keywords:** Phytochemical; Bitter kola; GC-MS; FTIR

## Introduction

Generally, most countries make use of medicinal plant for home remedy with or without orthodox medicine [1]. These plants are easy to access as they are cultivated within residence and at farming areas; it is even a practice by some persons to grow medicinal plants of various type in their garden [2]. Most of these medicinal plants are grown due to their abilities to boost blood level, abilities to reduce inflammations, antidiabetics ability [3]. Research on medicinal plants has proven that plants with this unique ability all have one thing in common which is phytochemicals. It is the presence of phytochemicals that gives these plants their medicinal ability [4]. Alkaloids, phenols, tannins, terpenes and flavonoids are the major constituents of these plants. The rich presence of hydroxyl group in phenols provides them with their ability to stabilize unpaired electrons in radicals thus they are useful in treating ailments associated with free radicals [5]. Alkaloids cytotoxicity makes them useful in managing cancerous cells. Some of these plants have the ability to restore inflamed tissues due to the presence of flavonoids [6].

Garcinia kola is a perennial medicinal plant used in ethno-medicine for the treatment of several ailments, including diabetes. Plants are important to the sustenance of life; they regulate atmospheric gases, recycle matter in biogeochemical cycle, serves

as food to animal and humans for their growth and development [7]. Beyond nutritional role there are other functions that plant plays in helping humans and animals survive in their environment [8]. Plant secondary metabolites such as phytochemicals are used to fight pathogens [9]. These phytochemicals when consumed by animals and humans elicits protective action against pathogens, oxidative stress, inflammation and cancer [10].

Medicinal plants play a crucial role in our pharmaceutical industries and they have increased tremendously because of their ability to manage free radicals, their relative abundance and low cost [11].

This study focused on identifying the phytochemicals, bioactive compounds present in bitter kola leave, their *in-vitro* antioxidant activities and compared n-hexane, ethanol and aqueous solvent to identify effective solvent for extraction of these phytochemicals in relation to food, health and nutritional benefits.

## Materials and methods

### Collection of plant material and identification

Bitter kola leaves were taken in July 2021 from an open area in Akinyele Oyo State, Nigeria. Garcinia kola leaves were identified by Ibrahim BAKTIR, a plant taxonomist at Cyprus International University's Department of Botany. An herbarium accession number CIU-DB 0253 was deposited at the department's herbarium.

### Extract preparation

Fresh Bitter kola leaves were cleaned with distilled water, the dirt was removed, and the leaves were allowed to air dry for two weeks to acquire a constant weight before being ground manually into a coarse powder. In order to extract 100g of roughly crushed leaves with 400ml of various solvents (n-hexane, ethanol, and aqueous), cold maceration was utilized for 24 hours. Whatman No. 1 filter paper was used to filter the filtrate once more after the extracts had been filtered using fine-pore cheese cloth. The extract was then concentrated at 50 °C in a rotary evaporator for two hours before being moved to a water bath and dried off, producing a dark brown substance. The substance that was removed was put in a glass container and kept at 4 °C until needed.

### Phytochemical screening

#### Qualitative screening of phytochemical

Standard procedures were used to conduct preliminary phytochemical screening of the different leaf extracts of bitter kola, as reported by Dah-Nouvlessounon et al. [12], Sbhatu et al. [13] to screen for the presence of different chemical components (saponins, phlobatannins, cardiac glycosides, flavonoids, tannins, phenol, terpenoids, steroids, carbohydrates, and proteins) and to establish quantitative phytochemical determinations for total phenol and flavonoids by the method of Sulaiman et al. [14].

#### Quantitative analysis of phytochemical

#### Estimation of total phenols and total tannins

For three minutes, 1.0ml of each of the three bitter kola leaf extracts (aqueous, n-hexane, and ethanol) were mixed with 1.0ml of the Folin-phenol Ciocalteu's reagent. Next, 1.0ml of saturated $Na_2CO_3$ was added, and the volume was increased to 10ml by adding distilled water. The reaction mixture's absorbance at 725nm was measured after 90 minutes in complete darkness. With different concentrations of gallic acid and tannic acid (20-100g/ml), a calibration curve for phenol and tannin was made. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extracts and milligrams of tannic acid equivalents (TAE) per gram of extracts, respectively [15].

#### Estimation of total flavonoids

Test tubes containing 1.25ml of distilled water and 0.075ml of a 5 percent $NaNO_2$ solution were combined with 0.5ml of aqueous, n-hexane, and ethanol extracts from bitter kola leaves respectively. For five minutes, the tubes were left to sit. After 6 minutes, 0.15ml of 10% $AlCl_3$ and 0.5ml of 1.0M NaOH were added, and the solutions were then diluted with 0.275ml of distilled water. The absorbance at 510nm was immediately determined. The standard used was catechin (20-100g/ml). The amount of flavonoids in total was calculated as milligrams of catechin (CAE) equivalents per gram of extracts [16].

#### Reducing sugar determination assay

In a gently covered test tube, 1.5ml of DNS reagent was added to 1.5ml of several bitter kola leaf extracts (aqueous, n-hexane, and ethanol) because a simple test tube was used, the test tube was coated with a piece of paraffin film to prevent liquid loss due to evaporation. To generate the red-brown hue, the mixture was heated to 90 degrees Celsius for 5 to 15 minutes. The color was then stabilized using 0.5ml of a 40 percent potassium sodium tartrate (Rochelle salt) solution. The absorbance was measured using a spectrophotometer at 575 nm after cooling to room temperature in a cold-water bath. The total reducing sugar content was expressed as milligram of glucose (GluE) equivalent/g extracts (200-1000µg/ml) [17].

#### Total protein determination

Protein concentration of sample extract was determined by means of the Biuret method as described by [18]. 1ml of Bitter kola leaf extract filtrates was mixed with 3ml of Biuret reagent. After 30 minutes of incubation at room temperature, the absorbance was measured at 540nm using distilled water as a blank. The protein content of the samples was estimated from the BSA standard curve using bovine serum albumin (BSA) (200-1000g/ml) as a reference protein.

#### Quantitative Estimation of Alkaloids

To 1ml of test leaf extracts of bitter kola, 5ml pH 4.7 phosphate


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buffer, 5ml BCG solution, and 4ml chloroform were mixed together and shaken. The extracts were collected in a 10ml volumetric flask and diluted with chloroform to adjust volume. The complex's absorbance in chloroform was measured at 470 nm in comparison to a blank made in the same way but without extract. The test was compared to Atropine equivalents (40-120g/ml) using Atropine as a reference material [19].

### Determination of phytate

One millilitre of extracts samples was vortexed thoroughly with 0.4 mM HCl followed by centrifugation at 3000rpm for 5 minutes. With distilled water, the final volume was increased to 1.0ml from the 0.1ml supernatant. It was then given 1.0ml of the color reagent (3M sulfuric acid, 2.5 percent w/v ammonium molybdate, and 10% ascorbic acid mixed in equal volume with two equal volumes of distilled water) and left at room temperature for one hour. A spectrophotometer was used to measure optical density against a blank at 650nm. The standard was potassium dihydrogen phosphate [20].

## In-Vitro antioxidants activities

Determination of antioxidant activity and free radical scavenging of leaf extracts of Garcinia kola to 2ml of DPPH solution, 0.2ml of various doses of bitter kola leaf extracts (1.0-7.0mg/ml) were added (0.3mM). The absorbance was measured at 517nm after 30 minutes of dark incubation [21].

### Nitric oxide (NO) free radical scavenging activity

Two milliliters of 10mM sodium nitroprusside dissolved in 0.5 milliliters of 10 mM phosphate buffer saline (pH 7.4) were combined with 0.5 milliliters of various amounts of bitter kola leaf extracts (1.0-7.0mg/ml). After that, the mixture was incubated at 250°C. After 150 minutes of incubation, 0.5ml of the incubated solution was removed and mixed with 0.5ml of Griess reagent [1.0ml sulfamic acid reagent (0.33 percent in 20% glacial acetic acid at room temperature for 5 minutes with 1ml naphthyl ethylene diamine dichloride (0.1 percent w/v)]. After 30 minutes at room temperature, the mixture's absorbance was measured at 546nm against a blank [1].

### Reducing power assay (RP)

To 1.0ml of different leaf extracts of bitter kola (1.0-7mg/ml), 2.5ml of 0.2 M phosphate buffer (pH 6.6) and 2.5ml of $K_3Fe(CN)_6$ (1 percent w/v) were added. After a 20-minute incubation at 500°C, 2.5ml trichloroacetic acid (10% w/v) was added to the mixture. The top layer of the solution (2.5ml) was collected by centrifugation at 3000 rpm for 10 minutes, then mixed with distilled water (2.5ml) and 0.5ml $FeCl_3$ (0.1 percent, w/v). After that, the absorbance was compared to a blank sample at 700nm (that contained distilled water and sodium phosphate buffer) [22].

### Determination of total antioxidant capacity

In screw-capped tubes, 1.0ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added and dissolved, followed by 0.1ml of different bitter kola leaf extracts. The tubes were sealed and incubated for 90 minutes at 95°C in a thermal block. The absorbance of the aqueous solution in each tube was measured at 695nm against a blank after cooling to room temperature. Gallic acid (20-100g/ml) was used as a standard, and total antioxidant capacity was measured in gallic acid equivalents (GAE) [23].

## FTIR analysis

The determination and identification functional groups of various types was analysed using FTIR (Fourier-Transform Infrared Spectroscopy) [24]. The chemical bond in FTIR is as a result of wavelength light rays absorbed. The molecule contains bonds which are determined by reading the FTIR. Furthermore, 10mg of extracts were transferred to an agate motor for toiling and KBr (pellet) of 100mg was added for encapsulation leading to the formation of sample disc (translucent) which was pestle in other to form a powdered sample which is used for FTIR analysis.

## GC-MS analysis

The extracts of bitter kola leaves were analyzed through GC–MS (SHIMADZU QP2010) analysis to determine the active compound. 1µl of extracts were injected into the GC. The capillary column (30m × 0.25µm × 0.25µm) was being used in the following conditions: oven temperature coded from 80°C for 1min, then progressively increased at 280 °C at 5 min; injector temperature (250 °C), carrier gas (Helium), flow rate is 1ml /min; the volume of the injection sample was 1µl; split ratio 1:0; ionization energy 70eV: Run time 28min. The relative quantity of every component was computed by the comparison between its average peak area to the total area. The Identification details of the separated volatile compounds was carried out through retention indices and mass spectrometry by comparing using database of National Institute Standard and Technology (NIST), library 2008.

## Statistical analysis

All of the data was statistically analyzed. The mean and standard deviation of the values were recorded, and a one-way ANOVA was performed to test for differences between treatment groups. P-values of less than 0.05, or at the 95 percent confidence level ($p<0.05$), were deemed significant. For statistical comparisons, the Turkey HSD post-hoc tool was employed.

# Results and discussion

## Percentage yield of leaf extracts of bitter kola

The percentage yield of the aqueous, n-hexane and ethanol


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leaf extract of bitter kola were 20.5% w/w, 7.7% w/w and 8.5% w/w respectively. The yield was a dark brown mass of 20.5 g for aqueous extract, dark green masses of 7.7 g for n-hexane and 8.5 g for ethanol respectively extracts.

## Qualitative Phytochemical screening of leaf extracts of bitter kola

**Table 1:** Phytochemical Screening of leaf extracts of *bitter kola.*

| Phytochemicals | Aqueous Extract | n-hexane Extract | Ethanol Extract |
|---|---|---|---|
| Saponins | - | - | - |
| Tannins | + | + | + |
| Terpenes | - | - | - |
| Steroids | - | - | - |
| Alkaloids | + | + | + |
| Phlobatannins | - | - | - |
| Cardiac glycosides | - | - | - |
| Flavonoids | + | + | + |
| Phenols | + | + | + |
| Carbohydrates | + | + | + |
| Proteins | + | + | + |
| Key + = Present, - = Absent | | | |

The results of the qualitative phytochemical screening of leaf extracts of bitter kola are presented in Table 1. The outcome showed the presence of tannin, phenol, alkaloid, flavonoid, protein and carbohydrate in all leaf extracts of aqueous, n-hexane and ethanol respectively. While Cardiac glycosides were detected both in ethanol and n-hexane leaf extracts.

## Quantitative Phytochemical determination of leaf extracts of bitter kola

**Table 2:** Quantitative Phytochemical determination leaf extracts of *Garcinia kola*.

| Phytochemicals | Aqueous Extract | N-hexane Extract | Ethanol Extract |
|---|---|---|---|
| Phenols (mg/gGAE) | $31.2 + 2.14^{a}$ | $51.0 + 0.79^{b}$ | $145 + 4.00^{c}$ |
| Flavonoids (mg/gCAE) | $4.37 + 0.13^{a}$ | $19.1 + 0.29^{b}$ | $82.6 + 4.90^{c}$ |
| Tannins (mg/gTAE) | $36.1 + 2.76^{a}$ | $58.1 + 0.03^{b}$ | $178 + 5.13^{c}$ |
| Alkaloids (mg/gATE) | $0.59 + 0.03^{a}$ | $2.09 + 0.02^{a}$ | $28.9 + 1.01^{b}$ |
| Reducing sugars (mg/gGluE) | $815 + 8.00^{a}$ | $280 + 5.39^{b}$ | $469 + 2.67^{c}$ |
| Proteins (mg/g) | $0.02 + 0.01^{a}$ | $0.99 + 0.02^{b}$ | $7.91 + 0.04^{c}$ |
| Phytates (mg/g) | $5.39 + 0.19^{a}$ | $1.00 + 0.14^{b}$ | $6.90 + 0.30^{c}$ |
| Values are means + standard deviations of triplicate determinations. GAE = Gallic acid equivalent, CAE = Catechin equivalent, TAE= Tannic acid equivalent, ATE = Atropin equivalent, GluE = Glucose equivalent.Values not sharing common superscript on the same row differ significantly ($p<0.05$) | | | |

The results of the quantitative determination of leaf extracts of bitter kola are presented in Table 2. The result showed that the ethanol leaf extract of bitter kola recorded the highest concentration in total phenol, flavonoids, tannin, alkaloid, reducing sugar, protein and phytate when compared to both aqueous and n-hexane leaf extracts ($p<0.05$).

## Antioxidant Activities of leaf extracts of bitter kola

### Inhibition of DPPH by leaf extracts of bitter kola

The results of the *in-vitro* free radical scavenging activities of leaf extracts of bitter kola against 1, 1-diphenyl -2-picryl hydrazyl are presented in Figure 1. The results showed that the n-hexane leaf extract recorded the highest inhibitory activities when compared to both n-hexane and ethanol extracts ($p<0.05$) across all concentrations. However, all extracts showed increase in inhibitory activities with increasing concentrations.

### Inhibition of nitric oxide of leaf extracts of bitter kola

The results of the inhibition of nitric oxide by leaf extracts of bitter kola are depicted in Figure 2. The results revealed that the nitric oxide scavenging activities of the various extract of Garcinia kola were decreasing with increasing concentrations. However, the aqueous leaf extract recorded the highest inhibitory activities when compared to both n-hexane and ethanol extracts at 0.5mg/ml ($p<0.05$).


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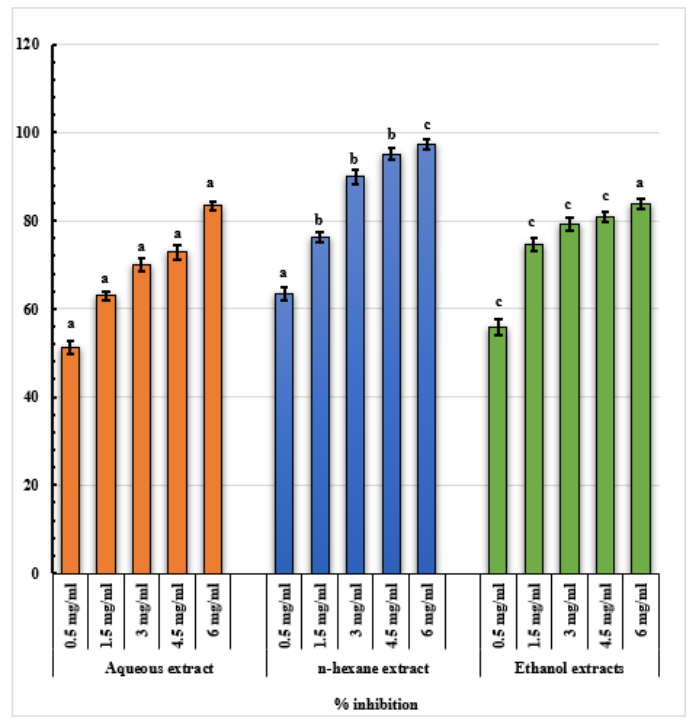


**Figure 1:** Inhibition of DPPH by leaf extracts of bitter kola Values are means + standard deviations of triplicate determinations. Values not sharing common superscript on the same row differ significantly ($p<0.05$).

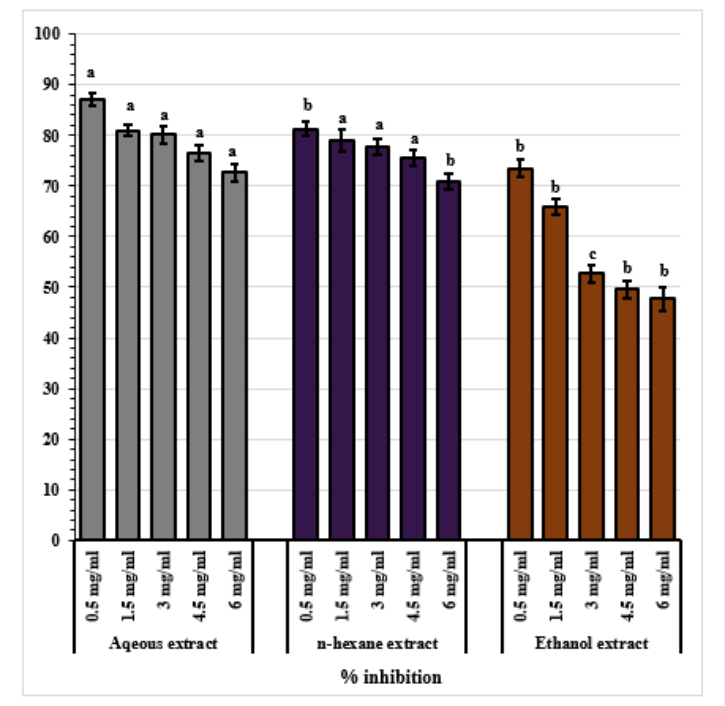


**Figure 2:** Inhibition of nitric oxide of leaf extracts of bitter kola Values are means + standard deviations of triplicate determinations. Values not sharing common superscript on the same row differ significantly ($p<0.05$).



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### Ferric reducing antioxidant power of leaf extracts of bitter kola

The results of ferric reducing antioxidant power of leaf extracts of bitter kola are presented in Figure 3. The result depicted that the reducing power potentials were increased with concentrations and the aqueous leaf extract obtained the highest reducing properties from 4.5mg/ml to 6mg/ml when compared to both n-hexane and n-hexane leaf extracts ($p<0.05$).

### Total antioxidant capacity of leaf extracts of bitter kola

The results of total antioxidant capacity of leaf extracts of bitter kola are embodied in Figure 4. The results showed that the aqueous leaf extract recorded the highest activities when compared to both n-hexane and ethanol extracts ($p<0.05$) from 0.5mg/ml to 6mg/ml (30.1 ± 1.01 to 82.4 ± 1.07mg/gm GAE).

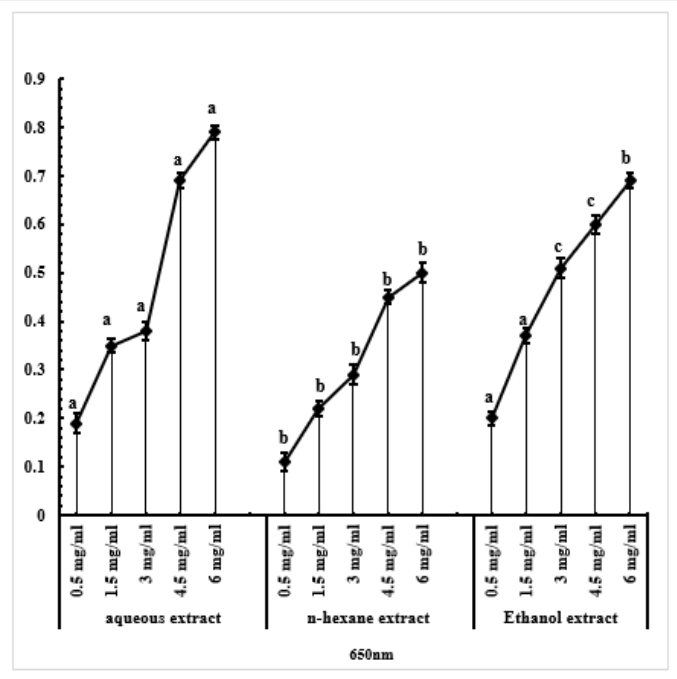


**Figure 3:** Ferric reducing antioxidant power of leaf extracts of bitter kola Values are means + standard deviations of triplicate determinations. Values not sharing common superscript on the same row differ significantly ($p<0.05$).

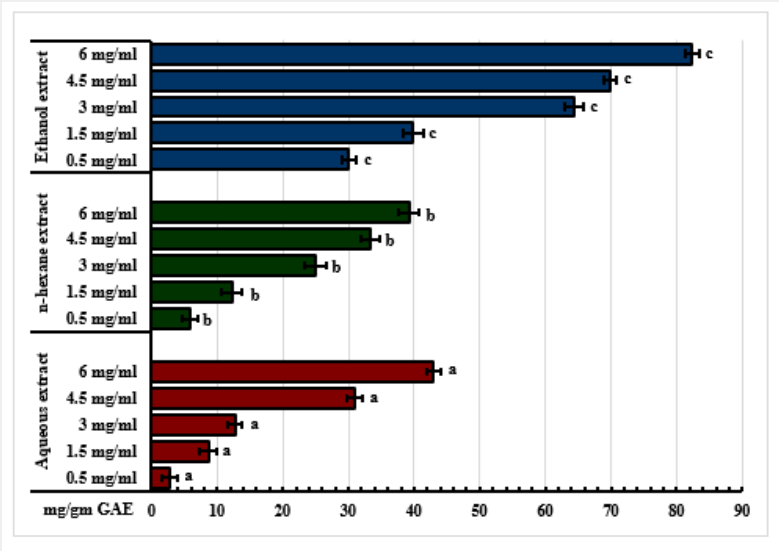


**Figure 4:** Total antioxidant capacity of aqueous leaf extract of bitter kola Values are means + standard deviations of triplicate determinations. Values not sharing common superscript on the same row differ significantly ($p<0.05$).



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Bitter kola leaves phytochemical screening indicated the presence tannins, alkaloids, phenols, carbohydrates and proteins in aqueous, n-hexane and ethanol extracts of the plant. The presence of tannins in plants gives them the abilities to function as antimicrobial and antiviral agents [25]. Tannins structure prevents virus from being absorbed into the blood stream and they are flushed out of the system [26]. Tannins antimicrobial action is elicited indirectly via limitation of substrate for microbial growth or inhibition of extracellular microbial enzymes [27]. Tannins exhibits cardioprotective properties through minimising calcification of glutaraldehyde-fixed aortic wall, preclusion of elastin degradation and stabilization of pericardial tissue [28]. Studies on human cancer cells has shown that tannins are more cytotoxic than phenols and this is due to the fact that they have galloyl groups in addition to hydroxyl group found in phenols [29]. Tannins have antidiabetic and antiobesity potential by preventing the intestinal absorption of glucose, α-amylase and α-glucosidase activities [30]. Tannins are known for anti-inflammatory and wound- healing properties and many laboratory tests confirmed that they have the ability to inhibit hyaluronidase and elastase enzymes [31]. Naturally found alkaloids are basic in nature and are biological active in diverse sites in the body [32]. The alkaloid atropine has antidepressant effect, relation of smooth muscle with its vasodilation done by the alkaloid [33]. Other important pharmacological activities of alkaloids are anti-hypertensive, anti-inflammatory, antioxidant, and hepatoprotective activity [34]. Bitter kola is used for its ability to keep people awake; the plant might serve to relief pain because of its alkaloid composition.

The presence of flavonoids in all three extracts of bitter kola indicates that the plant could serve as protective agent against bacteria and viruses, used as an antioxidants and anticancer agent [35]. Flavonoids scavenge free radicals by donating hydrogen to reactants thereby making them stable and preventing them from generating other reactants and attacking macromolecules, they also inhibit free radicals generation enzymes such as glutathione-S-transferase, NADH oxidase, microsomal monooxygenase [34]. Flavonoids can regenerate damage liver, research has proven their regenerative ability in carbon tetrachloride induced liver damage etc., hepatic dysfunction can be corrected when flavonoid is administered [35]. Based on the flavonoid content of bitter kola in this study, the plant leaves can be used in combating infections [36]. Chalcones, flavanol glycosides, isoflavones, galanin and genin are types of flavonoids with antibacterial activity, these flavonoids antibacterial effect is achieved through their binding with microbial proteins which hinders microbial activities [37]. Flavonoids can also act as analgesic and anti-inflammatory agent. Onions contains flavanol quercetin which plays a positive role in cancer formation, research on bitter kola could be extended to cancer studies [38]. Phenol is toxic to bacterial; they form complexes with proteins thereby making receptors unavailable for bacterial adhesion [39]. Their antioxidant role has been reported in numerous studies, they are important in fighting free radicals associated diseases. Bitter kola based on our result contributes to the energy pool of consumers, they supply proteins that will used in generating the required enzymes and receptor needed for body functioning. Proteins and carbohydrates found in this plant will be metabolized and incorporated into various structural components such as bio-signaling, membrane components etc.

The results of the quantitative analysis of bitter kola leaf extracts revealed that the ethanol leaf extract had the greatest content of total phenol, flavonoids, tannin, alkaloid, reducing sugar, protein and phytate when compared to both aqueous and n-hexane leaf extracts ($p<0.05$), ethanol seems to be a better extracting solvent for the plant and is expected to be more potent in antioxidant, antimicrobial, anti-inflammatory etc. activities. The results showed that the n-hexane leaf extract recorded the highest inhibitory activities for 1, 1-diphenyl -2-picryl hydrazyl which increased with concentrations while the plants leaves ability to inhibit nitric oxide decreased as the concentration of plant (0.5mg/ml to 6mg/ml), aqueous extracts showed more inhibitory effect than the other extracts. Aqueous extracts also had higher ferric reducing antioxidant power which increased with increase in concentration, When comparing the overall antioxidant capacity of bitter kola leaf extracts, the aqueous leaf extract had the greatest activity when compared to the n-hexane and ethanol extracts ($p<0.05$) from 0.5mg/ml to 6mg/ml (30.1 ± 1.01 to 82.4 ± 1.07mg/gm GAE).

Antioxidants are substances that stabilizers free radical, vitamin E, glutathione and vitamin C. These antioxidant acts at different level to scavenge free radicals, repair damage caused by these free radicals while others even prevent the damage from happening [40]. Bitter kola is used traditionally to treat infertility in males, prevent sepsis, serves as an aphrodisiac or used to cure cough, dysentery, chest colds, liver disorders, diarrhoea, laryngitis, bronchitis, and gonorrhoea [41]. Bitter kola can protect the liver from induced damage [42]. Most research done has focused on the seed part of bitter kola, phytochemicals present in the seed based on previous studies are tannins, saponins, alkaloids, cardiac glycosides [43]. This research is focused on evaluating phytochemical chemical component of bitter kola leaf extract .

## GC-MS

GC-MS chromatogram remains the best instrument for the separation of organic chemical compounds and identification of these compounds by means of mass spectroscopy. Under the current research, bioactive compounds found in extracts were identified (Table 3-5). Figure 5-7 were the chromatogram of extracts. Five compounds were identified in the ethanolic extract of bitter kola leaves. The compounds (Table 4a) were camphene (monoterpenes) at a retention time of 5.33 with a peak area of 43.2 which is known for its analgesic and anti-inflammatory property [7]. Eucalyptol (1,8-cineole) with a retention time of 14.67 and peak area of 7.6 is used as an antioxidant, anti-cancer and analgesic in drug formulation [8]. Trans-caryophyllene at


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retention time 18.161 with a peak area of 2.7 has an anti-microbial and anti-bacterial activity [44]. Limonene with a retention time of 19.832 and peak area of 1.2 has antimicrobial and antibacterial activity [9]. The GC-MS results obtained from the aqueous extract of bitter kola leaves were similar to those reported by [45]. Furthermore, the bio-compounds obtained in the present study were similar to the previous results reported by Ahsan et al. [46] who identified 20 components and also found that trans-anethole (65.59%), Trans-caryophyllene (13.11%), limonene (8.54%) and Eucalyptol (7.76%) which serves as hypocholesterolemic, antimicrobial, antitumor, immunosuppressant, antioxidant, nematicide and antiproliferative agent.

**Table 3:** Bioactivities of phytocomponents identified in the ethanol extract of bitter kola leaves by GC-MS.

| Retention Time (Min) | Compounds Name | Peak Area % | Pub Chem CID |
|---|---|---|---|
| 5.337 | Camphene (monoterpenes) | 43.2 | 6616 |
| 8.233 | 1,1,3,3-tetramethyl | 11.4 | 5945 |
| 14.671 | Eucalyptol (1,8- cineole) | 7.6 | 2758 |
| 18.161 | Trans-caryophyllene | 2.7 | 5354499 |
| 19.832 | Limonene | 1.2 | 22311 |

**Table 4a:** Bioactivities of phytocomponents identified in the n-hexane extract of bitter kola leaves by GC-MS.

| Retention Time (Min) | Compounds Name | Peak Area % | Pub Chem CID |
|---|---|---|---|
| 5.62 | 2,6-octadienal 3,7-dimethyl | 0.45 | 8843 |
| 12.9 | Docosane | 0.25 | 12405 |
| 14.457 | 1,2-Benzenedicarboxylic acid | 24.7 | 1017 |
| 18.652 | Gamma terpinene | 2.12 | 7461 |
| 19.942 | 2H-Pyran-3-ol | 0.87 | 22143352 |

**Table 4b:** Bioactivities of phytocomponents identified in the aqueous extract of bitter kola leaves by GC-MS.

| Retention Time (Min) | Compounds Name | Peak Area % | Pub Chem CID |
|---|---|---|---|
| 9.928 | 2 Cyclohexene-1-one | 0.76 | 13594 |
| 12.729 | Trimethylpyrazine | 0.94 | 26808 |
| 16.749 | 1 Dodecanol (n-dodecanol) | 0.54 | 12215322 |
| 19.594 | Hexadecamethyl | 0.3 | 10912 |
| 18.645 | octadecane | 0.23 | 70095 |
| 14.134 | 1,2-benzene dicarboxylic acid | 24.43 | 1017 |

**Table 5:** Absorption peak and functional group of bitter kola leaves ethanol extract.

| Absorption ($cm^{-1}$) | Functional Group | Peak Appearance |
|---|---|---|
| 3299.54 | O-H Stretching (Alcohol) | Medium |
| 2922.423 | CH- Stretching (Alkane) | Strong |
| 2178.43 | C=O Stretching (Aldehyde) | Weak |
| 1584.83 | N-H Bending (Amine) | Medium |
| 585.86 | C-L Stretching | Medium |
| 1152.24 | C-O Stretching | Weak |

In n-hexane extract, the following compounds (Table 4b) were identified using the database. 2,6-octadienal 3,7-dim ethyl with retention time of 5.62 and peak area 0.45% is used as an anti-fungal agent and anti-cancer treatment [47]. Docosane with retention 12.9 and peak area 0.25% has antioxidant and anti-microbial properties [10]. 1,2-Benzenedicarboxylic acid (phthalic acid) with retention time of 14.45 and peak area of 24.7 is used as an antioxidant and antibacterial agents [47]. Gamma terpene with retention time 18.65 and peak area of 2.12% has been reported to have a potent antioxidant property [48]. 2H-Pyran-3-ol with retention time 19.94 and peak area of 0.8% serves as antibacterial, antimicrobial and antioxidant agent. The GC-MS results profile


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obtained from the n-hexane extract of bitter kola leaves were similar to those reported by [49] showing hepatoprotective, anti-inflammatory, antihistaminic, hypocholesterolemic, anti-eczemic and anti-arthritic activities. In aqueous extract (Table 5). The major compounds identified are 2 Cyclohexene-1-one at a retention time of 9.928 with a peak area of 0.76%, is used as an antioxidant, antibacterial agents used in drugs formulation. Trimethylpyrazine (TMP) with retention time of 12.729 with a peak area of 0.94% is used in the treatment of cardiovascular diseases, headache and vertigo.

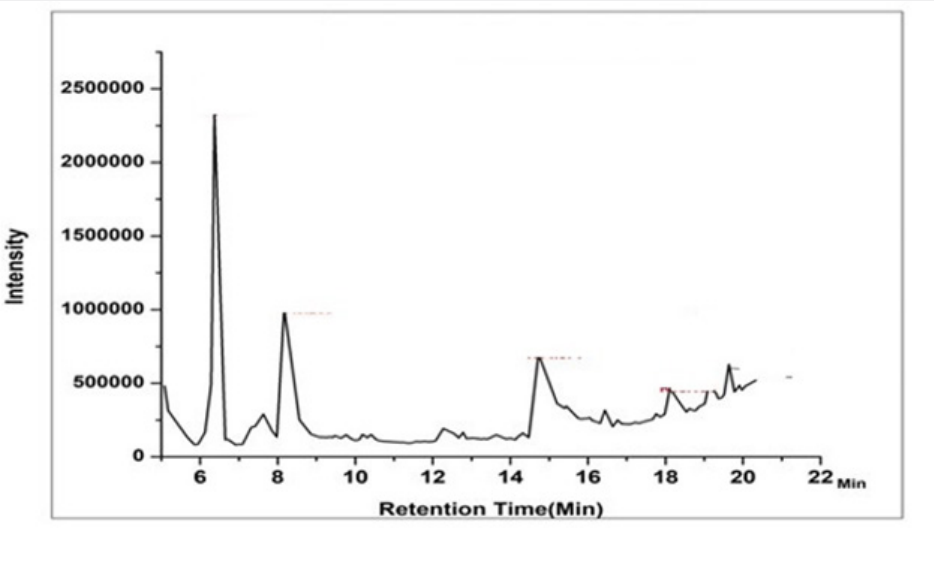


**Figure 5:** GC-MS chromatogram of ethanol extract of bitter kola leave.

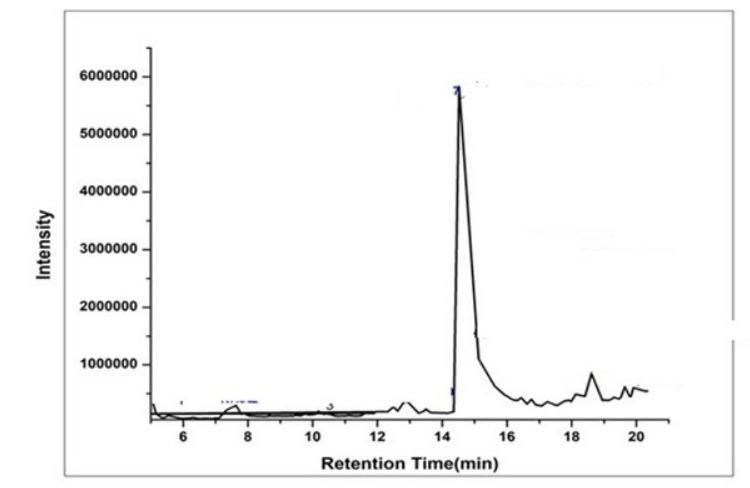


**Figure 6:** GC-MS chromatogram of n-hexane extract of bitter kola leave.

## FTIR spectroscopy

We confirm the presence of many characteristics functional groups as detected at different vibrational frequency band in the IR spectrum as shown in Figure 8 & 9. FTIR spectroscopic analysis (Table 6) of bitter kola leaves Figure 10 ethanol extract at 3299.54cm$^{-1}$ which is a representative of alcohol functional groups. The band peaking at 1584.83cm$^{-1}$ is of N-H bending (amine group) may be due to amino acids in the sample. Also, the spectrum observed at 2178.43cm$^{-1}$, 1152.24cm$^{-1}$ and 2922.423cm$^{-1}$ are due to the bond vibration of the carboxylic acid group. C-O stretching and aldehyde group respectively [50]. Similar results were previously reported by [51] which revealed the FTIR spectra


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peaking at 3414.50cm$^{-1}$, 2929.63cm$^{-1}$, 2854.70cm$^{-1}$ and 1453.40cm$^{-1}$ corresponding to O-H stretching of alcohol, carboxylic acids and C-N stretching respectively. The FTIR spectroscopic analysis of n-hexane extract (Table 7) displayed absorption bands 3288.63cm$^{-1}$, 2993.83cm$^{-1}$, 1500.23cm$^{-1}$ and 1057.85cm$^{-1}$ respectively [24]. The fingerprint at 3288.63cm$^{-1}$ in bitter kola leaves n-hexane extract which is the representative for O-H group predict the presence of alcohol in the sample, and we also confirm the strong band at 2993.83cm$^{-1}$, 1500.23cm$^{-1}$ and 1057.85cm$^{-1}$ are due to C-H Stretching (Alkane), N-O stretching (Nitro compounds) and C-O Stretching (Primary alcohol) respectively [45]. In these results of FTIR spectroscopic analysis of aqueous extract revealed the existence of various functional groups in the crude leave sample (Table 8). The IR spectra was recorded, and data are presented as broad peak spectrum at 3321.40cm$^{-1}$ representing OH stretching assigned to alcohol functional groups. Peak at 2981.63 and 1609.63cm$^{-1}$ is due to CH and C=C stretching representing alkane and alkene groups respectively. Peak at 3. 1498.73cm$^{-1}$ is due to N-O stretching (nitro compounds) [52].

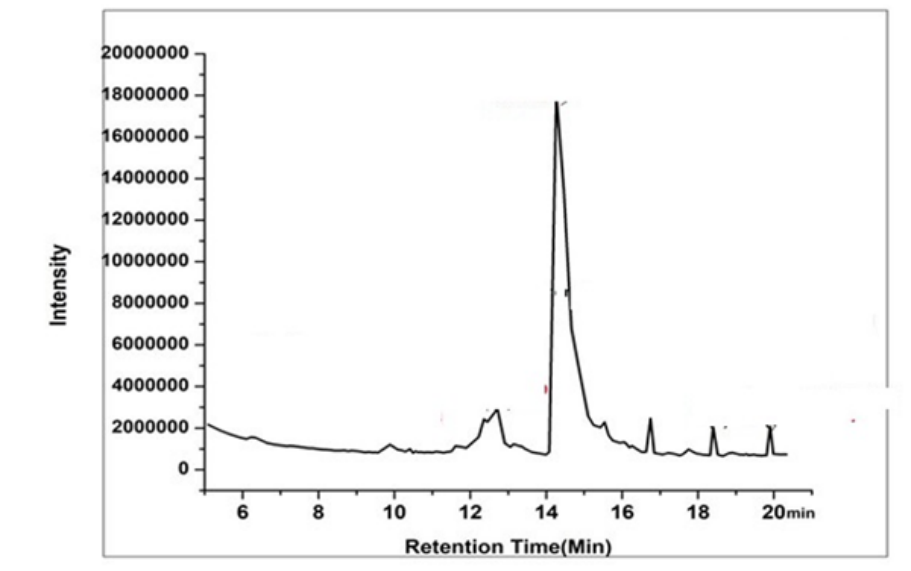


**Figure 7:** GC-MS chromatogram of aqueous extract of bitter kola leave.

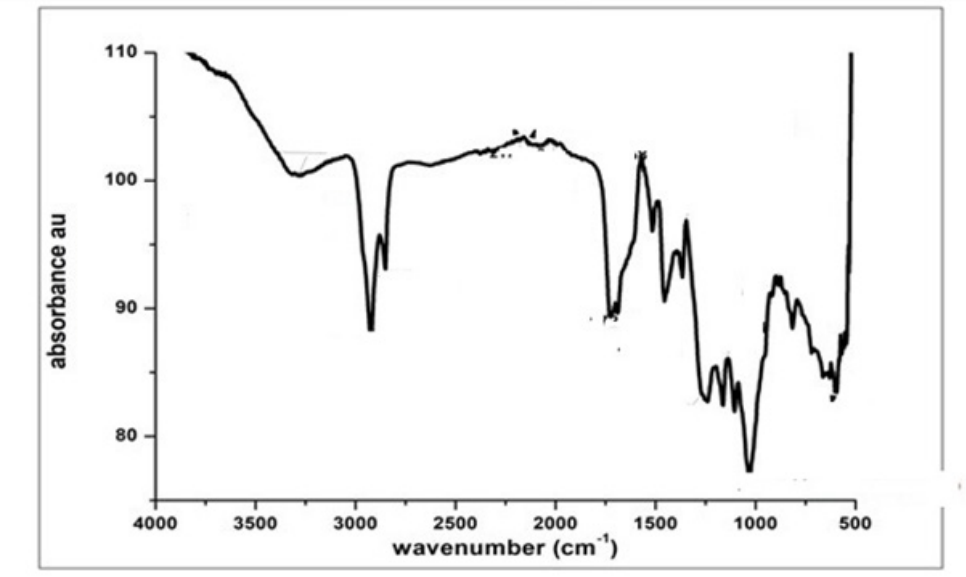


**Figure 8:** FTIR Spectrum of bitter kola leaves ethanol extract.


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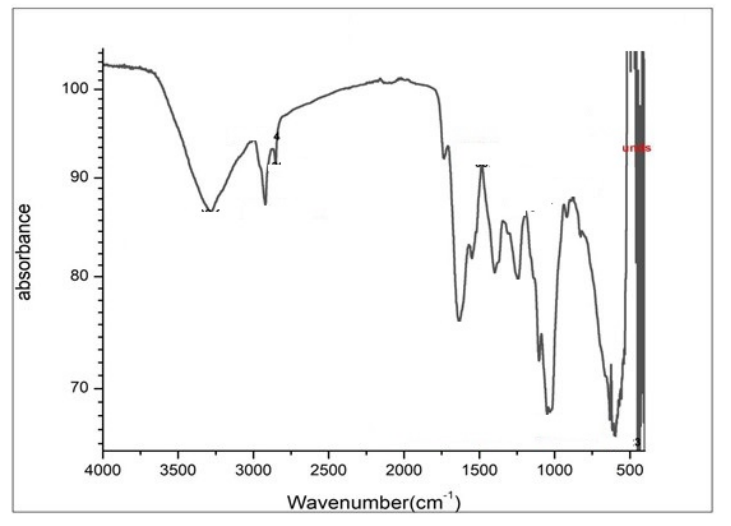


**Figure 9:** FTIR Spectrum of bitter kola leaves n-hexane extract.

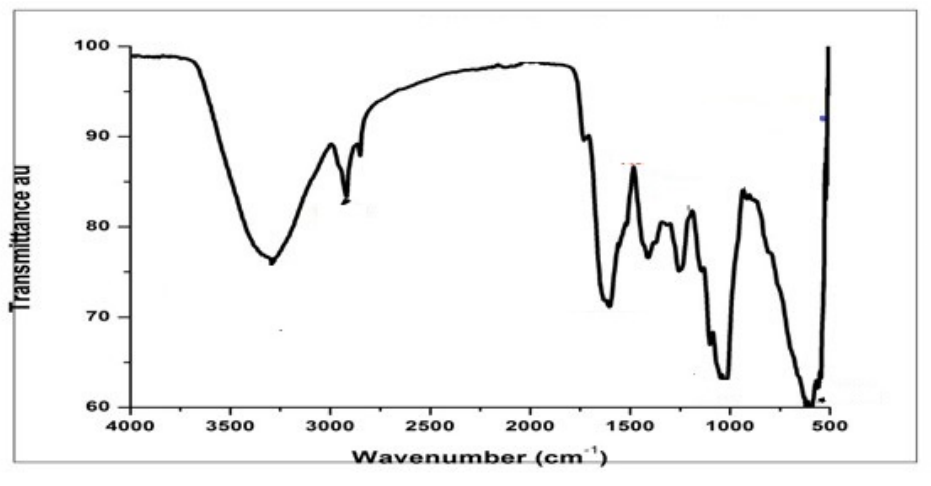


**Figure 10:** FTIR Spectrum of bitter kola leaves aqueous extract.

**Table 6:** Absorption peak and functional group of bitter kola leaves n-hexane extract.

| Absorption ($cm^{-1}$) | Functional Group | Peak Appearance |
|---|---|---|
| 3288.63 | O-H Stretching (Alcohol) | Strong |
| 2993.83 | C-H Stretching (Alkane) | Strong |
| 1500.23 | N-O stretching (Nitro compound | Medium |
| 1057.85 | C-O Stretching (Primary alcohol) | Strong |



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**Table 7:** Absorption peak and functional group of bitter kola leaves aqueous extract.

| Absorption ($cm^{-1}$)) | Functional Group | Peak Appearance |
|---|---|---|
| 3321.4 | OH Stretching (alcohol) | Strong |
| 2981.63 | CH stretching(alkane) | Medium |
| 1609.63 | C=C stretching (alkene) | Strong |
| 1498.73 | N-O Stretching (nitro compounds) | Strong |

## Conclusion

In conclusion, tannin, alkaloid, phenols, carbohydrates and protein are present in the leaves of bitter kola. Aqueous extraction of bitter kola is the best means of utilizing phytochemicals present in the plant this is based on the aqueous extracts inhibitory action on nitric oxide, ferric reducing antioxidant activity and total antioxidant capacity of leaf extracts. It should be noted that n-hexane was more effective when it came to inhibiting 1, 1-diphenyl -2-picryl hydrazyl. Consuming bitter kola leaf would boost the exogenous antioxidants of the body and help prevent and manage allergy, inflammations and other issues linked with oxidative stress. An abundance of secondary metabolites could certainly show up encouraging results in further pharmacological activities.

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## Author Contribution

Great Iruoghene EDO: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft, Visualization, Resources, Writing - Review & Editing, Supervision, Project administration

Favour Ogheneoruese ONOHARIGHO: Conceptualization, Methodology, Validation, Formal analysis, Investigation.

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