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Qualitative and Quantitative Phytochemical Screening of Cola Nuts (*Cola Nitida And Cola Acuminata*)

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Abstract

A study was carried out to evaluate the phytochemical constituent ofaqueous and methanol nut extracts of Cola*nitida* and *Colaacuminata*. The phytochemical screening which involves both the qualitative and quantitative analysis revealed the presence of secondary metabolites; alkaloid, tannins, glycoside, steroids and saponins glycoside with higher contain in methanol and aqueous extracts. The saponins content is higher in the aqueous extract and not detected in the methanol extract of the two species. Flavonoids expressed strong presence in methanol extract of *C.nitida* and not detected in the aqueous extract as well as the aqueous and methanol extracts of *C. acuminata*. For cardiac glycoside and volatile oil, they show moderate and trace presence in the methanol and aqueous extracts respectively. Anteraquinones are not detected in all the extracts of the two species. The quantitative result shows that, there were significant differences ($p<0.05$) in glycoside, tannins, saponins and alkaloid content of *C. acuminata* compared to *C.nitida*, *withC. acuminata* having the highest percentage of alkaloid (1.00%) while *C. nitida*had (0.80%) alkaloid content,*C. acuminata*had (0.80%) saponins, with *C. nitida* having (0.40%), more over the tanninscontent of *C. acuminata*was higher (0.89%) compared to *C. nitida*with (0.77%) while the glycoside content of *C. acuminata*was (0.53%) and that of *C. nitida was* (0.43%). The results as indicated above disclose the scientific basis for the traditional uses of cola nut. Finally, the study recommend the isolation of these metabolites using highly purify methods in other to obtain their maximum therapeutic potentials.

Keywords:*Colanitida, Cola acuminate*,Qualitative and Quantitative PhytochemicalScreening.

1.0 Introduction

Cola is a genus of about 125 species of trees indigenous to the tropical rain-forest African region (Ratsch, 2005). Phylogenetic information reveals that, the genus was formerly classified in the family Malvaceae, subfamily Sterculioideae and was later transferred into the separate family Sterculiaceae. The genus *Cola* contained five species of edible nuts - *Cola nitida* (important for trade), *Cola acuminata* (important for socio-cultural values), *Cola ballayi*, *Cola verticillata* and *Cola sphaerocarpa*. The latter three species are not known to be cultivated. The mature fruit of *Cola* species is a nut known as kola nut (Duke, 2001). It has a bitter flavour and high caffeine content (Benjamin *et al*., 1991and Blades, 2000). It is chewed in many West African cultures individually or in a group setting. It is often used ceremonially, presented to tribal chiefs or to guests. Chewing kola nut can ease hunger pangs. Kola nuts are used mainly for their stimulant and euphoriant qualities. They have effects similar to other xanthine containing herbs like cocoa, and tea. However, the effects are distinctively different, producing a stronger state of euphoria and wellbeing (Benjamin *et al*., 1991). They have stimulant effects on the central nervous system and heart. Kolanuts are used as a source of alkaloids in pharmaceutical preparations (Newall*et al*., 1996).

Kola pod husk has also been utilized for the production of liquid soap. The most recent and remarkable advancement in kola by- product utilization is the used of kola pod husk in the replacement of up to 60% of the maize used poultry feeds formulation (Bradley, 1992).

Phytochemical analysis of extracts from root, stem and seed of *Garcinia kola* and other members of the genus show that they contain reasonable amounts of phenolic compounds including biflavonoids (GB-1,GB-2), xanthones and benzophenones (Okunji*et al.*, 2007; Okoko, 2009). Their antibacterial activities are due to flavonoids especially biflavonoid type GB1 (Hong-xi and Song, 2001) and this has been demonstrated using methicillin-resistant *Staphylococcus aureus*(MRSA), vancomycin- resistant *enterococci* (VRE) (Han *et al*., 2005), *Lactobacillus* spp. (Owoseni and Ogunnusi, 2006) and *Streptococcuspyogenese*(Ogbulie*et al.*, 2007). (Afolabi*et al.*, 2008) has shown its antibacterial effects on *Streptococcus mutans*another important organism involved in

plaque formation. However, few reports are available on the antibacterial activities of *G. kola* on oral anaerobic species(Ndukwe*et al.,* 2005). Plant as a reservoir of effective chemotherapeutic agent can provide valuable natural drug and pesticides for effective and efficient management of human and plant disease. Some of the products of higher plants have been shown to be effective source of chemotherapeutic agent and provide renewable source anti-microbial infections of bio-degradable nature which are devoid of side effect (Farombi, 2003).

The study is aimed to determine the phytochemical constituent of water and methanol extracts of *Cola nitida* and *Cola acuminate*. The objectives of the research are:-

(i) To carried out qualitative analysis on the water and methanol extracts of *Cola nitida* and *Cola acuminate*.

(ii) To quantify the identified secondary metabolites in (i) above through quantitative phytochemical screening.

(iii) To compare the parameters interms of their content inthe two species.

1.1 Composition and Uses of Kola Nut

Kola nut mostly contains the following chemical composition; caffeine (2–3.5%), theobromine (1.0–2.5%), theophylline phenolics, phlobaphens, epicatechin, D-atechin, tannic acid, sugar, cellulose and water (Sanibare*et al.,* 2009; Anon, 2011). Kola nuts contain caffeine, which may explain their popular use in energy tonics and they are also thought to enhance male potency. Kola is reported to have aphrodiasic, stimulant, and cardiotonic properties (Benjamin *et al.,* 1991). In the Kitchen, the kola nut was once the main ingredient in coca-cola drinks. It has effects similar to other xanthine containing herbs like cocoa, tea etc. However, the effect is distinctively different, producing a stronger state of euphoria and wellbeing (Benjamin *et al*., 1991). The caffeine present acts as a bronchodilator, expanding the bronchial air passages (Jayeola, 2001; Kim, 2001). Kola nuts are also employed in the treatment of malaria and fever (Odugbemi, 2006). Experiments using animals indicate that kola nuts have analeptic and lipolytic properties and stimulate the secretion of gastric juices (GRIN, 2007). Odugbemi (2006) reported that the leaves of *Cola millenii* were used in the treatment of ringworm, scabies, gonorrhoea, dysentery and opthalmia. Kola nut is used as masticatory stimulant by Africans and has numerous uses in social, religious and rituals functions by natives in the forest region of Africa. It is used during ceremonies related to marriage, child naming, installation of chief's, funerals and sacrifices made to the various gods of African pynology. Various medicinal and pharmacological values have been observed in species of *Cola* (Steinegger and Hansel, 1992;Daels-Rakotoarison*et al*., 2003). Traditionally, the leaves, twigs, flowers, fruit follicles and the bark of *Cola nitida* and *Cola acuminata* are used to prepare a tonic as a remedy for dysentery, coughs, diarrhoea, vomiting and chest complaints (Burkill, 1995).

1.2 Propagation and Management

1.2.1 Cultivation

Ripe fruit harvested the follicles split open, the seed or nuts are extracted from the follicles and the white aril removed after 5days of fermentation.Yield of 300 nuts per tree are considered good. Nuts for planting are the mature ones that have undergone after ripening. Cola nitida can also be propagated by cutting or aerial layering. The seedlings are sometimes raised in pots or in polyethene bags, before planting.Outfield spacing of 10x10m are common. Early weeding is essential and inters planting with a shade tree is recommended. Initial growth is slow, reaching only 3m in 4 years. Slashing the trunk of kola tree before the season of main flowering at 4-5years and very few fruits can be obtained, but full production occur in 20years(Evans and Trease, 1999).

1.2.2 Weeding

Regular wedding is necessaryand this can either be done manually or by using herbicides. Some irrigation can be provided to the plants, but it is important to remove the water through an effective drainage system as excess water may prove to be detrimental for the growth of the plant when not grown in adequate shade, the kola nut plant responds well to fertilizer; Usually, the plants need to be provided with wind breaks to protect them from strong gales(El-Olemyl*et al.,* 1994).

1.2.3 Harvesting

Kola nut can be harvested by hand, by plugging it at the tree branch like in western countries and other countries of the world; it has been harvested by the use of harvesters(Evans and Trease, 1999).

1.2.4 Storage

Seed generally have recalcitrant storage behaviour seed can be retained for 1 year or more without loss in viability with seeds wrapped in banana leaves in basket, or with polyethene bags, at room temperature. Nut may be thus stored for several months without spoiling but with require regular changing of the leaves, and

checking for weevil damage(Evans and Trease, 1999).

1.3 Phytochemical Constituents of Kola nut

The kola nut tree including its leaves, twigs, nuts and bark contain several chemical constituents such as alkaloids, tannins, flavonoids, saponins, steroids, glycosides and reducing sugars.

1.3.1Alkaloids

These are organic nitrogenous compounds that have complex molecular structures of good pharmacological activity thus, bitter in taste and mostly basic in nature (El-Olemy*et al.,* 1994). These chemicals comprise up largest single class of secondary plant substances which contain one or more nitrogen atoms usually in combination as part of cyclic system. Alkaloids do not have an exact nomenclature but they are named as proto alkaloids, when they are without heterocyclic ring in their structures, pseudo alkaloids are those alkaloids with and without heterocyclic rings that are not derived from amino acids and the carbon skeleton is soprenoid, and true alkaloids, which are those that have heterocyclic rings in their structure. True alkaloids and photo alkaloids almost always have amino acids as their distal biosynthetic precursors and acetate is also incorporated in their structure. Alkaloids accumulate in actively growing tissues, epidermal and hypodermal cells, vascular sheath and latex vessels (Evans and Trease, 1999).

Alkaloids are usually colourless, often optically active substance, most are crystalline but few are liquid at room temperature e.g. nicotine. The alkaloid quinine for example is one of the prodominant bitter substances known, and is significantly bitter at molar concentration of 1 X 10^5. The most common precursors of alkaloids are amino acids. Many alkaloids are terpenoids in nature and some (e.gSolanine, the steroidal alkaloid of the potato) are the best considered from the biosynthetic point of view as modified terpenoids. Others are aromatic compounds e.g. colchicines. Alkaloids are rich in the angiosperms families and are generally absent or infrequent in the gymnosperms, ferns and lower plants (Evans and Trease, 1999).

1.3.2 Tannins

The term tannins denote substances present in plant extracts which are able to combine with proteins of animal hides and convert them into leather. Tannins are widely distributed in plants and occur in solution in the cell sap, often in distinct vacuoles. Tannins are readily soluble in water or alcohol, given as stringent solution that is useful in medicine. They are also used with ferric chloride in compound inks of greenish black to bluish black colours. There are two main groups of tannins; namely, true tannins and pseudo tannins. The true tannins are complex phenolic compounds. They display the general properties of tannins and are precipitated by gelatin in a 1% aqueous solution. True tanninsare further classified into two main classes; hydrolysable (pyrogallol) tannins (Ellagitannin and gallitannin), and condensed tannins (catechol and catechin). The pseudo tannins (gallic and ellagic acids) are simple phenolics that give some of the tests of tannins, but are not precipitated by gelatin. Tannins have a therapeutic value as astringents, since; they are able to precipitate proteins. Through this effect they can be used to stop hemorrhage and to treat diarrhea as well as local burns (EL-Olemy*el al*., 1994).

1.3.3 Flavonoids

These are the largest group of naturally occurring phenols and they occur in the plant both in the freestate and as glycosides. The flavonoids group may be described as a series of C_6-C_3-C_6 compounds. The majority of flavonoids are characterized by containing linkage of the three carbon chain with one of the benzene rings. Flavonoids are widely distributed in nature, but are more common in the cell sap of higher plants. They usually constitute the yellow, red and blue pigments of flowers and fruits. Flavonoids are important in many industries such as fermentation of tea, tannins of. Some flavonoids have fungicidal properties and are found to protect the plant against attack by pest and parasite (Evans and Trease, 1999).

1.3.4 Saponins

Saponins are one of the groups of glycosides found in many plant species with known foaming properties when mixed with water, allowing the formation of small stable bubbles. The amount of foam created by the crushed plant samples shaken with water in a jar is a good indication of the amount of saponins present. Saponins are normally broken down in the digestive system and are toxic when absorbed into the blood stream. They are used in modern times in the manufacture of fire extinguisher foam, tooth paste, shampoos, liquid soap and cosmetics. It is also used to increase the foaming of beer soft drink. As glycosides they are hydrolysed by acids to give an agylcone (sapogenin) and various sugar and related uronin acids. The steroidal saponin and pertocyclicterpenoids have a glycosydial linkage at -C_3 and have a common biogenetic origin through malvalonic acid and isoprenoid unit (Evans and Trease, 1999).

1.3.5 Glycosides

Glycosides are non-reducing substances, which on hydrolysis with reagents or enzymes yield one or more

reducing sugars among the products of hydrolysis. The non-sugar part of the molecule is called the agylcone or genin, and the sugar component, the glycone. The usual linkage between the sugar and agylcone is an oxygen linkage, connecting the reducing group of a sugar and an alcoholic or phenolic hydroxyl group of the agylcone. Such glycosides, sometimes called O-glycosides, are the most numerous ones found in nature. Other glycosides however occur, e.g. S-glycosides and N-glycosides in S-glycosides, e.g. Sinirin, where the sugar is linked to the thiol group of the agylcone. In n-glycosides (e.g. streptidine moiety of streptomycin and glucosamine), the sugar is linked to the amino group of the agylcone. There are also c-glycosides (e.g. barbalion) in which the sugar is linked to the agylcone by a carbon to carbon bond. All naturally occurring glycosides are of the β-type, although the α-linkage is found in some carbohydrates such as sucrose, glycogen and starch (Evans and Trease, 1999).

Glycosides occur widely in nature and occur in low concentration in nearly all plants. They occur not only in angiosperms but also in lower plants e.g. in streptomyces species. Glycosides are found in all parts of the plant, in roots, bark, leaves, flowers, fruits and seeds. Much plant pigments responsible for the colour of flowers and fruits are glycosides. Glycoside formation may well be a method of storing certain organic compounds e.g. phenols. It was also suggested that, some glycosides have a role of defence against the invasion of the tissue by micro-organisms subsequent to wounding, since many agylcones are aseptic and hence bactericidal in character. Plant glycosides that are currently used in medicine, though not larger in number, are important drugs. Glycosides of medicinal plants may be used as cardial stimulants (e.g. digitoxin and quabian or laxatives) Sinnosides and barbaloin or local irritants e.g. sinigrin or analgesics (silicon) and against capillary frugility (hesperidin) (Evans and Trease, 1999).

2.0 Materials and Methods

2.1 Samples collection and preparation

Five kilograms (5kg) each of *C. nitida*and *C. acuminata* were purchased at Sokoto Central market. The nuts were identified and authenticated by the curator of herbarium in the Department Biological Sciences, Botany unit of the UsmanuDanfodiyo University, Sokoto, where the voucher specimens were deposited. The nuts were washed with tap water and air dried under the shade. Dried materials were grounded to fine powder using pestle and mortar and kept in a sterile plastic bag at temperature of 25± 2^0C for further use.

2.2 Preparation of extracts

One hundred (100g) of each *Cola nitida* and *Cola acuminata* were separately placed in a round bottom flask containing one litre each of water and methanol. This was followed by mixing and agitation for six hours and it was allowed to stand for 24hours. The mixtures were filtered using muslin cloth and concentrated into powder by subjecting to heat using hot plate; the powdered dry extracts were scrapped offusing spatula. The dried extracts obtained were used directly for qualitative phytochemical sceening.

2.3 Phytochemical screening of plant extracts

2.3.1 Qualitative phytochemical screening

Phytochemical screening was conducted to qualitatively determine the presence or absence of the following secondary metabolites that is, Alkaloids, Tannins, Flavonoids, Saponins, Anthraquinones, Glycosides, Steroids, Saponins glycosides, Cardiac glycosides Volatile oil, and Reducing sugars, using the method outlined by (Evans and Sofowora, 1993; El-olemy*et al*., 1994; Siddiqui and Ali, 1997; Trease, 1999).

2.3.1.1 Alkaloids

Exactly 0.5g of each extracts were stirred with 10 cm^3 of 10% hydrochloric acid and allowed to stand overnight and then divided in to two parts for the following test.

a. 2 drops of Meyer's reagent were added to 1 cm^3 of the extracts, appearance of a creamy precipitate was taken as the evidence of the presence of alkaloids.

b. 2 drops of Wagner's reagent were added to 1 cm^3 of the extracts. A reddish brown precipitate observed in each test tube indicated the presence of alkaloids (Evans and Trease, 1999).

2.3.1.2Tannins

Few drops of $FeCl_3$ (%w/v) solution were added to 3 ml of the extracts in a test tube followed by shaking. A dirty green or dark blue coloration confirmed the presence of tannins (Evans and Trease, 1999).

2.3.1.3Flavonoids

One millilitre of the extracts was treated with 1 ml of dilute NaOH. The presence of a cloudy precipitate confirms the presence of flavonoids. (Evans and Trease, 1999).

2.3.1.4Saponins

a. Five (5ml) of distilled water were added to 2 ml of the extracts in a test tube and shaken vigorously. The formation of foams or stable frothing following the shaking indicated the presence of saponins. (Evans and Trease, 1999).

b. 0.5g of each extracts were separately shaken with distilled water in a test tube, followed by heating in a water bath to boiling point. Persistent frothing on warming confirms the presence of saponins (Sofowora, 1993).

2.3.1.5 Test for cardiac glycoside

(Keller-killiani's test) to one of herb extract 2 ml of 3.5% ferric chloride solution is added and allowed to stand for one minutes. 1ml of conc. H_2SO_4 was carefully poured down the wall of the tube so as to form a lower layer. A reddish brown ring at the interface indicated the presence cardiac glycoside.

2.3.1.6 Anthraquinones

Two milliliters of 10% hydrochloric acid were added to the extract in a test tube and boiled for about two minutes. Equal amount of chloroform was added to the test tube and vortexes twice, the chloroform layer was pipette out and then equal volume of 10% ammonia was added. A pinkish red colour observed in upper layer indicated the presence of anthraquinones (Evans and Trease, 1999).

2.3.1.7 Glycosides

Exactly 2.5ml of 50% sulphuric acid was added to 5ml of the extract in a test tube. The mixture was heated in boiling water for 15 minutes, cooled and neutralized with 10% NaOH Then 5ml of Fehlings solution was added and mixture was boiled. A brick-red precipitate was observed which indicate the presence of glycosides (Evans and Trease 1999).

2.3.1.8 Saponin glycosides

To 2.5ml of the extract was added 2.5ml of fehing's solution A and B. A bluish green precipitate showed the presence of saponin glycosides (El-Oley *et al.*,1994).

2.3.1.9 Test for reducing sugars

One milliliter each of Fehling's solutions I and II were added to 2 ml of the aqueous solution of the extract. The mixture was then heated in a boiling water bath for about 5 minutes. The production of a brick red precipitate indicated the presence of reducing sugars.

2.3.1.10 Tests for volatile oils

One1ml of the fraction was mixed with dil. HCI. A white precipitate was formed which indicated the presence of volatile oils (Evans, 1980).

2.3.1.11 Steroids

Exactly 2ml of acetic anhydride were added to 0.5g water extract of each sample with the addition of 2ml H_2SO_4. A colour change from violet to blue or green indicated the presence of steroids (Evans and Trease, 1999).

2.4 Quantitative phytochemical screening

2.4.1 Determination of total Alkaloids

Fifty (50g) of powdered Kola nut seeds were extracted with litre of methanol: (1:1v/v) mixture and solvent evaporated. The resultant residue was mixed with 200ml of H_2SO_4 and partitioned with ether to remove unwanted materials. The aqueous then extracted with excess chloroform to obtain the alkaloid fraction. The chloroform extraction was repeated several times and the bulk of extract was concentrated to dryness. The alkaloid was weighed and the percentage was calculated with reference to the initial weight of the sample powder(Evans and Trease, 1999).

$$\% \text{ Alkaloid} = \frac{\text{Weight of Alkaloid residue}}{\text{Volume taken}} \times 100$$

2.4.2 Determination of Total Saponins

Fifty (50g) of powdered extract was placed in a 500ml flask containing 300ml of 50% alcohol. The mixture was boiled under reflux for (30 minutes) and was immediately filtered while hot through a coarse filter paper.

Two (2g) of charcoal was added, the content was boiled and filtered while hot. The extract was cooled (some saponins may be separated) and an equal volume of acetone was added to complete the precipitation of saponins. The separated saponins were collected by decantation and dissolve in the least amount of boiling 95% alcohol and filtered while hot to remove any insoluble matter (Evans and Trease, 1999).

The filtrate was allowed to cool to room temperature there by resulting in precipitation of saponins. The separated saponins were collected by decantation and suspended in about 20ml of alcohol and filtered. The filter paper was immediately transferred to a desiccator containing anhydrous calcium chloride and the saponins were left to dry. They were weighed with reference to the weight of extract used

$$\% \text{ saponins} = \frac{\text{weight of saponins residue}}{\text{volume taken}} \times 100$$

2.4.3 Determination of Tannins

Powdered samples (0.1g) were put into a 100ml conical flask and 50ml of distilled water was added. The flask was gently heated to boiling for 1 hour, and the filtrate was collected in a 50ml volumetric flask. The residue was washed several times and the combined solution made to the volume with distilled water. To 10ml of sample solution in a 50ml volumetric flask, 2.5ml of Folin - Denis reagent and 10ml of $NaCO_3$ solution were added and made to volume with distilled water. The same treatment was made to the all the samples and the flasks were allowed to stand for 20 minutes after which optical density was measured at 760 nm using spectrophotometer (Evans and Trease, 1999).

$$\text{Tannic acid (mg/ml)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard} \times \text{Conc. of standard}}$$

2.4.4 Determination of glycosides

One gram (1g) of the extract was extracted with 10ml 70% alcohol and the mixture filtered. From the filtrate, 8ml was transferred to a 100ml volumetric flask and the volume was completed to the mark with distilled water. 8ml of the mixture was added to8ml of 12.5% lead acetate (to precipitate resins, tannins and pigments). The mixture was shaken well, completed to the volume (100ml) with distilled water and filtered. 50ml of the filtrate was pipette into another 100ml volumetric flask and 8ml of 4.7% disodium hydrogen phosphate (Na_2HPO_4) solution was added to precipitate excess lead. The mixture was made up to the volume with distilled water and mixed. This was filtered twice through filter paper. Baljets reagent (10ml) was added to 10ml of the purified filtrate. A blank sample of 10ml of distilled water was also added to 10ml baljets reagent. The two solutions were allowed to stand for one hour (time necessary for maximum colour development) a blank of 20ml distilled water was used. The intensity of the colour was read at 495nm using spectrophotometer. The colour was stable for several hours (Evans and Trease, 1999).

$$\text{The percentage of glycosides} = \frac{A \times 100}{17} \text{ g \%}$$

Where A = the absorbance of the colour at 495nm.

2.5 Statistical Analysis.

Data obtained from the studies were subjected to statistical analysis using Statistical Package for social sciences (SPSS) version 16.0. Analysis of variance (ANOVA) was carried on the data and means were separated using Duncan Multiple Range Test (DMRT).

3. **Results and Discussion**

3.1 Results

3.1.1 qualitative Phytochemical Screening

The results of qualitative phytochemical screening of two the Cola nutsare presented in Table 1.

Table 1: Qualitative Phytochemical analysis of *C. nitida*and *C. acuminata*

Phytochemical constituents	*Aqueous extract*		*Methanol extract*	
	C. nitida	*C, acuminata*	*C. nitida*	*C. acuminate*
Alkaloid	+++	+++	+++	+++
Saponins	+++	+++	-	-
Tannins	+++	+++	+++	+++
Flavonoids	-	-	++	-
Glycoside	+++	+++	+++	+++
Steroid	+++	+++	+++	+++
Anteraquinones	-	-	-	-
Saponins Glycoside	+++	+++	+++	+++
Cardiac glycoside	+	+	++	++
Volatile oil	+	+	++	++

Key

+++ = present in high concentration

++ = moderately present

+ = trace

- = not detected

3.1.2 quantitative Phytochemical Screening

The results of quantitative phytochemical content of *C .nitida*and *C .acuminata*were presented in table 2.

Table 2: Quantitative phytochemical content of *C. nitida* and *C. acuminata*

Cola spp	Glycoside	Tannins	Saponins	Alkaloid
C. nitida	0.43 ± 0.00^{a}	0.77 ± 0.00^{a}	0.40 ± 0.00^{a}	0.80 ± 0.00^{a}
C. acuminate	0.53 ± 0.07^{b}	0.89 ± 0.023^{b}	0.80 ± 0.00^{b}	1.00 ± 0.00^{b}

Values = mean ± standard error of 3 replicates mean.Mean in a column with different superscripts are significantly different (P< 0.05).

3.2 Discussion

The phytochemical analysisof cola nuts *(C. nitida and Cacuminata)* extract reveals it's a rich source of bioactive compound in potential diseases management. The present study presents a systematic screening of phytochemical constituents of C. nitida and C. acuminata against *Rhizopusoryzae,Mucorrecemosus,Apergillusniger,Aspergillusfumigatus.*

The study acknowledged the presence of various secondary metabolites like tannins,flavonoids, saponins, glycoside, steroid, saponins glycocides, alkaloids, cardiac glycoside, and volatile oil in the cola nut.The qualitativeand quantitative analysis carried out indicated the medicinal importance of *C. nitida and C. acuminata*. From the qualitative phytochemical analysis of aqueous and methanol extracts of *C nitida*and *C. acuminata*as stated in (Table 1) below revealed the presence of alkaloid, saponins, tannins, flavonoids, glycoside, steroid,saponin glycoside, cardiac glycocide volatile oil and the absent of anthraquinones in all the extracts.

The result shows thatalkaloid, tannins, glycoside, steroids and saponins glycoside have higher content in methanol and aqueous extracts, while saponins content is higher in the aqueous extract and not detected in the methanol extract of the two species. For flavonoids they are moderately presence in methanol extract of *C.nitida* and not detected in the aqueous extract as well as the aqueous and methanol extracts of *C. acuminata*. For cardiac glycoside and volatile oil, they show moderate and trace presence in the methanol and aqueous extracts respectively. Anteraquinones are not detected in all the extracts of the two species. This may not be unconnected with difference in polarity and thus difference extractability. These finding is conformity with those by (Alier*oet al.*, 2008,Yesmin*et al.*, 2008) on extracts of scadoxusmultiflorus and calotropisprocera. The presence of bioactive compounds is an indication that *C. nitida and C. acuminata*has medicinal potentials, this is due to the fact that

each of the compound identified has one or more therapeutic usage, presence of this active compounds. Similar results were also obtained byNyananyo*et* al., (2010) on some Niger Delta plants who observed the presence of flavonoids tannins alkaloid and saponins in large quantity.

The quantitative result shows that, there were significant differences ($p<0.05$) in glycoside, tannins, saponins and alkaloid content of *C. acuminata* compared to *C.nitida, withC. acuminata* having the highest percentage of alkaloid (1.00%) while *C. nitida*had (0.80%) alkaloid content,*C. acuminata*had (0.80%) saponins, with *C. nitida* having (0.40%).More over, the tanninscontent of *C. acuminata*was higher (0.89%) compared to *C. nitida*with (0.77%) while the glycoside content of *C. acuminata*was (0.53%) and that of *C. nitida was* (0.43%). Among the four groupsinvestigated quantitatively from the aqueous and methanol extracts of C nitidaand C. acuminata alkaloid where found to be most abundant followed by tannins, saponins, glycosides were the lowest in concentration and all compound varied across the cola species. This finding is an agreement with the work reported by Malikharjuna*et al.*,(2007) and Nwoko Cha *et al.*, (*2011*) they reportedabundance of tannins, alkaloids saponins flavonoids and glycoside in *Strychnospotatorum* L.F.

4. Conclusion

From the analysis carried out in this research work, the result showed scientific basis for the traditional uses of the plant extracts in which phytochemical analysis revealed the presence of tannins, saponins, alkaloid, glycoside, Saponin glycoside, cardiac glycoside volatile oil and steroid.Preliminary qualitative test is useful in the detection of bioactive principles and subsequently may lead to drug discoveries and development.

5. Recommendations

Based on the result of the study, the following recommendations are one ward advanced.

1. The phytochemical substances present in *C. nitida* and *C. acuminata*should be isolated and purified to obtain their maximum therapeutic potentials.
2. Further research should be done with a view of stabilizing the active ingredient so that they can be prevented from losing their potency.

6. References

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