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The phytochemical constituents and relative antimicrobial activities against clinical pathogens of different seed extracts of *Cola nitida* (Vent.), *Cola acuminata* (Beauvoir) and *Garcinia kola* (Heckel) grown in South West, Nigeria

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Abstract
The aim of the study was to investigate the *phytochemical* properties and the relative antimicrobial activity of aqueous, ethanol and methanol seed extracts of *Cola nitida, Cola acuminata and Garcinia kola respectively.* The phytochemicals were screened by qualitative and quantitative methods. Three different solvents: aqueous, ethanol and methanol were used to extract the bioactive compounds from the three species of Kola Qualitatively analyzed phytochemical constituents in the seed extracts of the three *Cola* species included Alkaloids, tannins, flavonoids, steroids, terpenoids and cardiac glycosides. The microorganisms assayed for antimicrobial activity using the agar diffusion method were: the gram positive *Staphylococcus aureus* and *Bacillus* spp, the gram-negative *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* respectively. Studies on sensitivity patterns of zones of inhibition exhibited by the crude methanol, ethanol and aqueous extracts of *Garcinia kola, Cola nitida and Cola acuminata* nuts showed relative degree of inhibitory effects against the test microorganisms: *Escherichia coli, Staphylococcus aureus, Lactobacillus, Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The zones of inhibition for *Escherichia coli* was the highest with methanol extract of 36.0mm followed by *Klebsiella pneumonia* with 25.5mm followed by *Staphylococcus aureus* with 23.5mm, and *Pseudomonas aeruginosa* with 18.5mm and *Lactobacillus* with 17.5mm respectively. Highest zones of inhibition started from methanol, ethanol and aqueous, their diameter of inhibition increases with increase in their concentrations. The methanol preparation of *Garcinia cola* nut was found to exhibit more significant inhibitory action ($p<0.05$) against the test organisms than the ethanol and aqueous preparations of *Garcinia kola and those of C. nitida and C. acuminata. Escherichia coli* in both preparations gave widest zones of inhibition than other microorganisms. *Cola spp* extracts with methanol can be explored in the development of drugs against infectious diseases.

Keywords: Phytochemical properties, antibacterial activity, *Garcinia kola, Cola nitida, Cola acuminata,* drug development.

1. Introduction
Medicinal plants are successful natural sources for the treatment of various infectious diseases of human (Seanego, 2012**)** [55]. Scientists are focusing on discovering natural compounds from medicinal plants, with the aim of introducing new drugs which will be more effective than those available in the market (Parekh *et al*., 2006) [32]. Recent research has focused on natural plant product as alternatives for existing drugs for curing diseases in developing countries (Aiyegoro *et al*., 2007) [3]. They have formed the basis of sophisticated traditional medicine and make an excellent lead for new drug development (Newman *et al*., 2000) [41].
Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases (Sonibare *et al*., 2009) [57]. Nature has been a source of medicinal agents for thousands of years. Although advances have been made in pharmacology and synthetic organic chemistry, this reliance on natural products, particularly on plants, remains largely unchanged (Trevor, 2001) [60]. These chemical constituents have great potential for medicinal use and both traditional healers and pharmaceutical drug companies exploit them (Akinpelu and Onakoya, 2006) [4]. Medicinal plants have been used as an ancient tradition especially where modern drugs are not affordable or inaccessible (Njume *et al*., 2009) [42].
Even today, plants are the most exclusive source of drugs for the majority of the world and people in developing countries especially use traditional medicine for their primary health care (Palombo *et al*., 2001) [50]. The world health organization has reported that about 80 % of the world's population is depending on traditional medicine and herbal remedies continue to play a role in the cure of diseases (WHO, 2001) [63].

Plants have provided a source of inspiration of novel drug compounds as plant derived medicines have made large contributions to human health and well-being (Maiyo *et al*, 2010) [28]. Their role is two fold namely; they provide key chemical structure for the development of new antimicrobial drugs and also as a phytomedicine to be used for the treatment of disease (Abukakar *et al.,* 2008) [1].

Moreover, potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, barks, roots and crude plant drugs. Antioxidants are vital substances which protect the body from damage caused by free radical inducing oxidative stress (Ozsoy *et al.*, 2008) [49]. Therefore, many plants were used as a source of traditional medicine to treat various diseases and conditions (Adediwura *et al.*, 2011) [2].

It is well established that some plants contain compounds able to inhibit microbial growth (Evarando *et al.*, 2005) [21]. These plant compounds have different structures and different action when compared with antimicrobials conventionally used to control microbial growth and survival (Nascimento *et al.*, 2000) [37]. The potential antimicrobial properties of plants are related to their ability to synthesize by secondary metabolism several chemical compounds of relatively complex structures with antimicrobial activity, including tannins, phlobatannins, alkaloids, coumarins, cardiac glycosides, terpenes, phenylpropanes, organic acids, flavonoids, isoflavonoids and saponins (Evarando *et al.*, 2005; Matasyoh *et al.*, 2009) [21, 32].

The phytochemical constituents medicinal uses of kolanuts have been studied in a number of regions. Kolanut (*Cola spp*.) belongs to the S*teruliacea* plant family with over 20 species native to the Africa tropical rain forest (Okoli *et al*., 2012) [45]. *Cola nitida* and *Cola acuminata* are the most common *Cola* species used (Dah-Nouvlessounon *et al*, 2015a) [13].

Apart from these two species cited above, there is *Garcinia kola* (angiospermae) belonging to the *Clusiaceae* family (Dah-Nouvlessounon *et al*, 2015b) [14]. The seeds of *G. kola* are currently used in traditional medicine in many herbal formulations and have potential therapeutic benefits due largely to the activity of their flavonoids and other bioactive compounds (Dah-Nouvlessounon *et al*, 2015b) [14] These species are sources of caffeine in processing and pharmaceutical industries and often chewed by some ethnic's group settings as stimulants (Okoli *et al*., 2012) [45]. The presence of other chemicals in kola nuts such as kolanin and theobromine also makes them suitable for use in drug preparation (Dewole *et al*,2013) [17] In addition, research has shown some potential uses of kola nut in the production of wine, chocolate and many non-alcoholic beverages (Dah-Nouvlessounon *et al*, 2015c, 2015d) [15, 16].

On account of the emerging development of drug resistance by pathogenic microorganism against synthetic antibiotics; attention has now shifted to extracts of biologically active components isolated from plant species used as herbal medicine. Medicinal plants may offer a new source of antibacterial, antifungal and antiviral activities (Maiyo *et al*, 2010) [28]. Studies have shown that they have less side effects, less expensive and effective against broad spectrum drug resistant microorganisms (Newman *et al*., 2000; Parekh *et al*., 2006; Motamedi *et al*., 2010) [41, 52, 36].

It is in the light of this background that the study was designed specifically to determine and compare the phytochemical constituents and *in vitro* evaluation of the relative antimicrobial activity of the aqueous, ethanolic and methanolic seed extracts of *Cola nitida, Cola acuminata* and *Garcinia kola* grown in South West, Nigeria.

2. Materials and Methods

2.1 Samples Collection

Five kilograms (5kg) each of Garcina kola, *C. nitida* and *C. acuminata* freshly harvested was purchased from Eleyewo-daily-Market in Akungba-Akoko Ondo State, South West, Nigeria. The nuts were authenticated by the Curator of the Herbarium of Biological Sciences Department, Ahmadu Bello University, Zaria where the voucher specimens were deposited for reference. The nuts were washed with tap water and air dried under the shade, and later kept in an ambient container. They were thereafter transported to the Research Laboratory of Biochemistry Department, Kaduna State University (KASU) till commencement of the study.

2.2 Samples Preparation

The seed were cut into pieces and allowed to dry at room temperature. The dried materials were pulverized to fine powder using electric blender, and later sieved. The powdered preparations were kept in a sterile plastic bag at temperature of 25± 2 ºC for further use.

2.3 Preparation of Cola Extracts

Twenty grams of powdered plant materials mixed with 100ml solvents (Distilled water, Ethanol and Acetone solution). The *Cola spp* extracts preparations were done as previously described by Alade and Irobi (1993) [6]. The extracts were prepared by using soxhlet apparatus collected and stored in a vial for further studies.

2.4 Chemicals/ Reagents

All reagents used for this study were of analytical grade and supplied by a reputable chemical manufacturers in the purest form available. Meyer's reagent, Wagner's reagents, Hydrochloric acid, Sodium chloride, potassium ferrocyanide, Ferric chloride, sodium hydroxide Fehling solution, ethanol, methanol, acetic anhydride, were products of BDH Chemicals Ltd, Poole, England. Sulphuric acid, barium chloride, diethyl ether, n-butanol was procured from Sigma Chemical Co. St Louis, USA.

2.5 Collection of clinical isolates

Pure isolates of *Escherichia coli, Staphylococcus aureus, Lactobacillus, Klebsiella pneumonia, Pseudomonas aeruginosa* was collected and authenticated in Microbiology laboratory of Microbiology department, Kaduna State University Kaduna and the identity was reconfirm using appropriate biochemical test. For the experiments, the bacterial isolates were first subcultured in nutrient broth (Oxoid, Ltd) and incubated according to standard procedure.

2.6 Preparation of 0.5 McFarland standards

Solution A was prepared by adding 1.175g of barium chloride ($BaCl_2$, $2H_2O$) to 100ml distilled water. Solution B was prepared by adding 1ml of sulphuric acid (H_2S0_4) (0.36N) to 100 ml of distilled water. Then 0.5 ml of solution A was added to 99.5 ml of solution B, mixed well and distributed in test tubes with a screw cap. The cap is closed tightly to avoid evaporation. The mixture was stored in the dark. The solution was agitated vigorously before using it. After standardization of bacterial suspension, a sterile cotton swab was immersed in it and was rotated several times with firm pressure on the inside wall of the tube to remove excess fluid.

2.7 Phytochemical Screening of Plant Extracts

2.7.1 Phytochemical Screening

Phytochemical screening of extract was based on methods

described by Trease and Evans (1983) [59] with some modifications. In this method, phytochemical screening was conducted to qualitatively determine the presence or absence of the following phytochemicals in *C. nitida* and *C. acuminata* respectively. These are: Alkaloids, Tannins, Flavonoids, Saponins, Anthraquinones, Glycosides, Steroids, Saponins glycosides, Cardiac glycosides and Volatile oils. Quantitative screening was done in accordance with standard procedures (El-Olemy *et al*, 1994 and Harbone, 1998) [20, 23]

2.8 Pathogenic test

The isolates of *Escherichia coli, Staphylococcus aureus, Lactobacillus, Klebsiella pneumonia, Pseudomonas aeruginosa* were subjected to catalase and coagulase test using standard procedure.

2.8.1 Sensitivity testing of aqueous, ethanolic and methanolic extracts of C. nitida, C. acuminata and G. kola on bacterial isolates

The agar well diffusion technique of Kirby-Bauer was employed to test the antimicrobial effects of *Cola nitida, Cola acuminata* and *G. kola* extract as recommended by NCCLS(1987,2000) [38, 39].

2.9 Data Analysis

Data were analyzed using simple percentages, mean, standard deviation and student t-test at P< 0.05 using Statistical Package for Social Sciences (Version 13.0) software (SPSS, Chicago, IL.)

3. Results

Fig 1: *C. nitida* nuts

Fig 2: *C. acumiata*

Fig 3: *G. kola*

(Fig. 1, Fig. 2 and Fig. 3 pix adapted from Dah-Nouvlessounon *et al* (2015c) [15]

The analysis presented in Tables 1 and 2 shows the result of phytochemical constituents of the three species of kola nut (*Garcinia kola, Cola nitida* and *Cola acuminata)* extracted with aqueous, methanol and ethanol respectively. The table shows the solubility of these species in different solvent as well as the presence of phytochemicals like tannins, flavonoids, saponins, glycoside, steroid, saponins glycosides, alkaloids, cardiac glycoside, and volatile oil. Result has shown that qualitative and quantitative analysis carried out indicated that the three solvent used for extraction were very effective, with methanol being more effective in extracting more active compound as shown in table 2. This was followed by ethanol and aqueous respectively.

Qualitative phytochemical screeening of aqueous, methanol and ethanol extracts of *C. nitida, C. acuminata* and *Garcinia kola* revealed the presence of alkaloids, saponins, tannins, flavonoids, glycosides, steroid, saponins glycoside, cardiac glycoside volatile oil and the absence of anthraquinones in all the extracts. The result shows that alkaloid, tannins, glycoside, steroids and saponins glycoside have higher content in methanol, ethanol and aqueous extracts, while saponins content is higher in the aqueous extract and moderately present in methanol and ethanol extract of the three species. For flavonoids, they are moderately present in methanol and ethanol extract of *C. nitida* and predominantly present in *Garcinia kola*. and not detected in the aqueous extract as well as the aqueous ethanol and methanol extracts of *C. acuminata*, but also predominant in aqueous extract of *Garcinia kola*. For cardiac glycoside and volatile oil, they showed moderate and trace presence in the methanol, ethanol and aqueous extracts respectively. Anthraquinones was not detected in all the extracts of the three species (Table 1).

Result of quantitative analysis shows that there were significant differences ($p<0.05$) in tannins, saponins, flavonoid, phenol and alkaloid content of methanol, ethanol and aqueous extract of *Garcinia kola* compared to *C. acuminata* and *C. nitida* with the methanol extract of *Garcinia kola* having the highest percentage of Saponins: (13.06%), followed by *C. acuminata* (10.20%) and *C. nitida* had (10.01%). The ethanol extract of *Garcinia kola* has the second highest percentage of Saponins (12.02%), followed by *C. acuminata* (09.04%) and *C. nitida* (09.02%). The aqueous extract of *Garcinia kola* having the least percentage of Saponin (09.84%), *C. acuminata* (08.40%) and *C. nitida* had (08.10%).

Methanol extract of Alkaloid content, *Garcinia kola* had (0.49%) *C. acuminata* had (0.30%) and *C. nitida* (0.26%). Ethanol extract of Alkaloid content, *Garcinia kola* had (0.45%) *C. acuminata* had (0.26%) and *C. nitida* (0.23%). Aqueous extract of Alkaloid content, *Garcinia kola* had

(0.32%) *C. acuminata* had (0.26%) and *C. nitida* (0.20%).
Methanol extract of tannin with *Garcinia kola* (0.98%), *C. acuminata* (0.44%), *C. nitida* having (0.46%). Ethanol extract of tannin with *Garcinia kola* (0.44%), *C. acuminata* (0.32%), *C. nitida* having (0.38%). Aqueous extract of tannin with *Garcinia kola* (0.73%), *C. acuminata* (0.34%), *C. nitida* having (0.36%).
Methanol extract of flavonoid with Garcinia kola (0.68%), *C. acuminata* (0.46%), *C. nitida* having (0.49%). Ethanol extract of flavonoid with *Garcinia kola* (0.64 %), *C. acuminata* (0.43%), *C. nitida* having (0.47%). Aqueous extract of flavonoid with *Garcinia kola* (0.52%), *C. acuminata* (0.32%) and *C. nitida* having (0.32%) respective ely.
Moreover, the phenol content of methanol extract of *Garcinia kola* (0.42%), *C. acuminata* (0.31%) and *C. nitida* with (0.31%). Ethanol extract of *Garcinia kola* (0.15%), *C. acuminata* (0.28%) and *C. nitida* with (0.28%). Aqueous extract of *Garcinia kola* (0.40%), *C. acuminata* (0.29%) and *C. nitida* with (0.29%). Among the five groups of phytochemicals investigated quantitatively from the aqueous, methanol and ethanol extracts of the three Cola species, Saponins were found to be most abundant followed by tannins, flavonoid, alkaloid and phenol in varied concentration (Table 2)
The results on sensitivity patterns of Zones of Inhibition exhibited by the crude methanol, ethanol and aqueous extract of *Garcinia kola, Cola nitida and Cola acuminata,* nut possess some degree of inhibitory effects against the test microorganisms of *Escherichia coli, Staphylococcus aureus, Lactobacillus, Klebsiella pneumonia and Pseudomonas aeruginosa* respectively. From Table 3, there was a corresponding increase in the zones of inhibition of *Staphylococcus aureus* as the strength of 4mg/ml of 23.5mm methanol suspension of *Garcinia kola* nut per disc increases exhibits the highest inhibition followed by ethanol suspension of 4mg/ml of 20.5mm and aqueous suspension of 4mg/ml with 15.5mm. Same goes for 3mg/ml, 2mg/ml and 1mg/ml of the suspension respectively. *Escherichia coli* gave the widest zones of inhibition that ranged from 36.0 mm, of Methanol suspension of 4mg/ml, the Ethanol suspension 4mg/ml, with 21.5mm and aqueous suspension of 4mg/ml, with 20.0 mm. *Pseudomonas aeruginosa* ranged between 18.5 mm, 19.5 mm, 17.5 mm of the 4mg/ml of both methanol, ethanol and aqueous suspension. *Klebsiella pneumonia* gave wide zones of inhibition that ranged from 25.5 mm, of Methanol suspension of 4mg/ml, the Ethanol suspension 4mg/ml, with 18.0mm and aqueous suspension of 4mg/ml, with 18.5 mm. *Lactobacillus* gave wide zones of inhibition that ranged from 17.5 mm, of Methanol suspension of 4mg/ml, the Ethanol suspension 4mg/ml, with 19.0 mm and aqueous suspension of 4mg/ml, with 16.5 mm.
From Table 4, there was an increase in the zones of inhibition of *Escherichia coli, Staphylococcus aureus, Lactobacillus, Klebsiella pneumonia, Pseudomonas aeruginosa* with an increase in the weights of suspension. The zones of inhibition for *Staphylococcus aureus* as the strength of 4mg/ml of 18.0mm methanol suspension of C*ola nitida* nut per disc, ethanol suspension of 4mg/ml of 19.5 mm and aqueous suspension of 4mg/ml with 16.5mm respectively. *Escherichia coli* gave the wide zones of inhibition that ranged from 16.5 mm, of Methanol suspension of 4mg/ml, the Ethanol suspension 4mg/ml, with 20.0 mm and aqueous suspension of 4mg/ml, with 13.0 mm. *Pseudomonas aeruginosa* ranged between 18.0 mm, 21.0 mm, 14.0 mm of the 4mg/ml of both methanol, ethanol and aqueous suspension. *Klebsiella pneumonia* gave wide zones of inhibition that ranged from 21.0mm, of Methanol suspension of 4mg/ml, the Ethanol suspension 4mg/ml, with 20.0mm and aqueous suspension of 4mg/ml, with 15.5mm. *Lactobacillus* gave wide zones of inhibition that ranged from 16.5mm, of Methanol suspension of 4mg/ml, the Ethanol suspension 4mg/ml, with 15.5mm and aqueous suspension of 4mg/ml, with 14.0mm.
From Table 5, there was an increase in the zones of inhibition of *Escherichia coli, Staphylococcus aureus, Lactobacillus, Klebsiella pneumonia, Pseudomonas aeruginosa* with an increase in the weights of suspension. The zones of inhibition for *Staphylococcus aureus* as the strength of 4mg/ml of 19.5mm methanol suspension of C*ola acuminata* nut per disc, ethanol suspension of 4mg/ml of 20.0mm and aqueous suspension of 4mg/ml with 13.5mm respectively. *Escherichia coli* gave the wide zones of inhibition that ranged from 21.5mm, of Methanol suspension of 4mg/ml, the Ethanol suspension 4mg/ml, with 19.0mm and aqueous suspension of 4mg/ml, with 16.0mm. *Pseudomonas aeruginosa* ranged between 18.0mm, 20.0mm, 13.5mm of the 4mg/ml of both methanol, ethanol and aqueous suspension. *Klebsiella pneumonia* gave wide zones of inhibition that ranged from 19.0mm, of Methanol suspension of 4mg/ml, the Ethanol suspension 4mg/ml, with 18.0mm and aqueous suspension of 4mg/ml, with 15.0mm. *Lactobacillus* gave wide zones of inhibition that ranged from 22.5mm, of Methanol suspension of 4mg/ml, the Ethanol suspension 4mg/ml, with 26.5mm and aqueous suspension of 4mg/ml, with 12.5mm.

4. Discussion

The findings from present study has revealed the presence of alkaloid, saponins, tannins, flavonoids, glycoside, steroid, saponins glycoside, cardiac glycoside, volatile oil and the absent of anthraquinones in all the extracts of *Cola spp* (*C. nitida, C. acuminata* and *Garcinia kola*) in the aqueous, methanol and ethanol used as solvent. Solvent used for extraction were very effective, with methanol being more effective in extracting more active compound, followed by ethanol and water. Studies have shown that the type of solvents used has an effect on the nature of compounds extracted and the resulting bioactivity of the extract (Eloff *et al*., 1998; Arankuma and Muthuselvam, 2009; Seanego, 2012) [8, 19, 55]. This clearly implies that polarity of solvents (non-polar, polar and less polar) play a vital role in the extraction of bioactive compounds, which influence the antimicrobial activity (Houghton and Raman, 1998; Parekh *et al.,* 2005) [24, 51]. It is important for the efficiency of extraction to be optimized in order to ensure that many potential active constituents are extracted as possible. The rate of extraction, quantity extracted, handling of extracts, toxicity of solvent are some factors that need to be evaluated in order to ensure the value of a solvent. A variety of solvents are applied in the extraction of antimicrobial compounds (Masoko *et al*., 2007) [31].
Water being a universal solvent is more often than not used in preparation of folkloric medicine (Masoko *et al*., 2007) [31]. Natural products such as pigments and bioactive components are soluble in water which point out high yield of extract; while some solvents are only selective for certain bioactive compounds (Nwaokorie *et al.,* 2010) [43]. In our present study, the methanol extract was more promising as evidenced in the manifestation of high inhibitory activity against the clinical isolates. Generally, methanol extracted more active ingredients than ethanol and water. This may be due to the higher volatility of methanol compared to water and ethanol

as the highest extraction was observed in the methanolic extract of Saponins (13.06%). This finding is in conformity with those of earlier investigators (Seanago, 2012; Ukaoma *et al.,* 2013; Alaje *et al.,* 2014) [55, 62, 7].

The qualitative test used in the detection of phytochemicals shows that, all the extract posseses active metabolites in varied concentration of (>10%). Thus the aqueous, methanol and ethanolic extracts of *Cola spp* (*C. nitida, C. acuminata* and *G. kola respectively)* contained alkaloids,, tannins, saponins, flavonoids, glycosides and phenols. This we have found in our present study to be the active bioprinciple that confer antimicrobial properties to kola. This has also been confirmed in one of our related study on *C. nitida and, C. acuminata* respectively (Omwirhiren *et al.,* 2016) [47].

Alkaloids were high in the extract of *C. spp* and this tallied with findings of earlier researchers (Okoli *et al,* 2012; Okeke *et al.,* 2015) [45, 44]. Irvine (1961) [25] showed that the nuts of *C. nitida* grown in Ghana contained more caffeine. Caffeine from alkaloid chemotaxonomy is a well known pseudoalkaloid of plant origin containing a purine group in its structure. Most of the physiological actions of these kolanuts have been found to be due to its caffeine content (Okoli *et al*., 2012, Dah-Nouvlessounon *et al.,* 2015a, 2015b & 2015c) [45, 13-15]. Zenk and Junger (2007) [64] reported that caffeine has been among the social drugs consumed by human due to its stimulating effect, presumably. Caffeine has also been known as a fat burner and is beneficial in assisting weight loss (Outlaw *et al*., 2013) [48]. Hypoglycemic effect of *C. acuminata* has been observed by Adediwura *et al*., (2011) [2] suggesting that caffeine alkaloid exhibit antidiabetic property.

Also tannins have been implicated to have been pharmacologically used as astringents for precipitating proteins and related macromolecules like centriole as well as the filaments of microorganisms (Tyler *et al*; 1998; Chikezie *et al.,* 2008;Bartosz *et al;* 2012) [61, 10, 9]. Tannins bind to both proteins and carbohydrates and several other substances (Eleazu *et al*., 2012) [18].

Their presence can cause browning or other pigmentation in both fresh foods and processed products (Arankuma and Muthuselvam, 2009) [8]. The presence of tannin in *C. spp* implies they may among other properties quicken the healing of wounds and burns as it prevents wound from being septic (Sibanda *et al*., 2010; Ukaoma *et al*., 2013) [56, 62].

This probably account for their usage in herbal medicine, (Eleazu *et al.,* 2012) [18]. Tannins have been shown to exhibit high potency for the treatment of intestinal disorders such as diarrhea and dysentery (Akinpelu, *et al.,* 2008 and Sonibare *et al*., 2009) [5, 57].

The high Saponins content in *G. kola* substantiate its use as a good source of chemical substances with the potential therapeutic benefit. Saponin has the property of precipitating and coagulating the red blood cell and also binding bad cholesterol (Dewole *et al.,* 2013, Mir *et al.,* 2013 and Omwirhiren *et al.,* 2016) [17, 35, 47]. A substantial body of literature and works on this subject have advocated and supported the use of these plants in folklore medicine. *G. kola* is a good source of antibiotic as the active metabolites tested were very toxic which is of immense use as a defensive mechanism against pathogenic micro-organisms. This findings from present study conforms to previous ones (Okwu, (2003), Ukaoma *et al*., (2013),) [46, 62].

The moderate amount of saponin in Cola species investigated suggests that they may not be deleterious to the user as kolanut has been known to be a social fruit eaten by humans in all ages, (Johnson, *et al*., 1986 and Dah-Nouvlessounon, *et al.,* 2015c & 2015d) [27, 15, 16]. In addition, many plants contain non-toxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens (Jayalakshmi *et al*., 2011 and Temitope *et al.,* 2016) [26, 58].

The presence of these phytochemicals in cola species, most especially alkaloids and saponins explains the reason why these medicinal plants are used to treat hypertension because alkaloids and saponins prevent the excessive intestinal absorption of cholesterol and thus reduce the risk of cardiovascular diseases such as hypertension (Akinpelu, *et al.,* 2008) [5].

The aqueous, methanol and ethanol extracts of *C. nitida, C. acuminata* and *Garcinia kola* studied produced a good zones inhibition against the selected test micro-organisms (*Escherichia coli, Staphylococcus aureus, Lactobacillus, Klebsiella pneumonia, Pseudomonas aeruginosa).* The kola extract were very potent in terms of activity because of the relative presence of bioactive components. The broad spectrum of activity exhibited by *Cola spp* against gram positive and gram negative bacteria probably explains their use in a wide range of ailments in developing economies. The multiple antimicrobial resistance bacteria causes severe problem that results in complication of treatment of bacteria infections and this has been recognized by the World Health Organization, (WHO, 2001) [63]. Antibiotics are used and were believed to lead in the complete eradication of infectious diseases (Rosina *et al.,* 2009) [54]. Despite the progress made in introducing new antibiotics, the emergence of drug resistant strains cause failure of infectious disease treatment (Mathias *et al.,* 2000; Gibbons, 2005; Makhatsa, 2007; Maiyo *et al*., 2010) [37, 22, 29, 28]. Studies have shown that antibiotic resistance occur as a result of an intrinsic mechanism that prevent bacteria from destruction (Cohen, 1992, McDonnell *et al*., 2001) [11, 34]. These bacteria usually do not have the structural cellular mechanisim that are needed in order for the antibiotic to act upon (Courvalin *et al*., 2001, Mannetti *et al*.,2007) [12, 30]. Therefore the management of resistant bacteria is an attractive strategy using medicinal plants. Medicinal plants caters for about 80% of the vast populace that rely mostly on herbs for their medicines, (Arunkmar and Muthuselvam, 2009; Maiyo *et al*., 2010; Temitope *et al*., 2016; Neethu *et al*., 2016) [8, 28, 58, 40].

On account of the produced good inhibition zones against the test clinical isolates (*Escherichia coli, Staphylococcus aureus, Lactobacillus, Klebsiella pneumonia, Pseudomonas aeruginosa*), findings from present study therefore suggest that *Cola spp* from south west, Nigeria could be a promising potential therapy in the treatment infectious diseases caused by these organisms and if the active ingredients are isolated and possibly crystallized, therapeutic antibiotics could be produced from *Cola spp*.

Appendix of Results

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

Phytochemical constituents	Aqueous Extract			Methanol Extract			Ethanol Extract		
	C. acuminata	*C. nitida*	*Garcinia kola*	*C. acuminata*	*C. nitida*	*Garcinia kola*	*C. acuminata*	*C. nitida*	*Garcinia kola*
Alkaloid	+++	+++	+++	+++	+++	+++	+++	+++	+++
Saponins	+++	+++	+++	++	++	++	++	++	++
Tannins	+++	+++	++	+++	+++	+++	+++	+++	+++
Flavonoids	+	++	++	++	++	+++	++	++	+++
Glycoside	+++	+++	+++	+++	+++	+++	+++	+++	+++
Steroid	+++	+++	+++	+++	+++	+++	+++	+++	+++
Anthraquinones	-	-	-	-	-	-	-	-	-
Saponins Glycoside	+++	+++	+++	+++	+++	+++	+++	+++	+++
Cardiac glycoside	+	+	+	++	++	++	++	++	++
Volatile oil	+	+	++	++	++	++	++	++	++

Key

+++ = phytochemicals present in high concentration, ++ = phytochemicals moderately present, + = trace, - =, phytochemicals not detected
C. acuminata = (Cola acuminata), C. nitida = (Cola nitida) and G. kola = (Garcinia kola).

Table 2: Result of the quantitative Phytochemical Content of *Garcinia kola, C. acuminata*, and *C. nitida*

Samples	Solvent	Saponin	Flavonoid	Tannin	Alkaloid	Phenol
G. kola	Ethanol	12.02±0.00	0.64±0.02	0.44±0.01	0.45±0.01	0.15±0.003
	Methanol	13.06±0.10	0.68±0.04	0.98±0.02	0.49±0.00	0.42±0.000
	Aqueous	09. 84±0.04	0.52±0.01	0.73±0.00	0.32±0.00	0.40±0.007
C. Nitida	Ethanol	09.02±0.01	0.47±0.00	0.38±0.03	0.23±0.01	0.28±0.003
	Methanol	10.01±0.00	0.49±0.02	0.46±0.01	0.26±0.04	0.31±0.009
	Aqueous	08.10±0.12	0.32±0.00	0.36±0.02	0.20±0.01	0.29±0.006
C. Acuminata	Ethanol	09.04±0.02	0.43±0.00	0.32±0.07	0.26±0.10	0.28±0.032
	Methanol	10.20±0.10	0.46±0.01	0.44±0.00	0.30±0.08	0.31±0.069
	Aqueous	08.40±0.07	0.32±0.06	0.34±0.10	0.20±0.04	0.29±0.022

Results are mean ± standard deviation of triplicate determinations
Key: *C. acuminata* = (*Cola acuminata*), *C. nitida* = (*Cola nitida*) and *G. kola* = (*Garcinia kola*).

Table 3: Sensitivity patterns of Zone of Inhibition exhibited by the crude methanol, ethanol and aqueous extract of *G. kola* on some pathogens at different concentrations.

Bacteria	Solvent	Zones of Inhibition (mm*)			
		4mg/ml	3mg/ml	2mg/ml	1mg/ml
Staphylococcus aureus	Methanol	23.5	18.5	17.0	15.0
	Ethanol	20.5	16.0	13.5	12.0
	Aqueous	15.5	12.5	11.0	10.5
Escherichia coli	Methanol	36.0	29.0	25.0	19.0
	Ethanol	21.5	18.5	15.5	11.5
	Aqueous	20.0	18.0	15.5	12.5
Pseudomonas aeruginosa	Methanol	18.5	17.0	16.0	15.5
	Ethanol	19.5	17.5	16.0	11.5
	Aqueous	17.5	12.0	11.5	10.5
Klebsiella pneumonia	Methanol	25.5	24.5	19.5	18.0
	Ethanol	18.0	13.5	11.5	11.0
	Aqueous	18.5	14.0	12.0	11.5
Lactobacillus	Methanol	17.5	13.5	12.0	11.5
	Ethanol	19.0	17.5	16.0	10.5
	Aqueous	16.5	15.5	12.5	11.0

Key: mm = millimeters, mg/ml = milligram per millimeter, *G. kola = Garcinia kola*
* Result significant @ P, 0.05

Table 4: Sensitivity patterns of Zones of Inhibition exhibited by the crude methanol, ethanol and aqueous extract of *C. nitida* on some pathogens at different concentrations.

Bacteria	Solvent	Zones of Inhibition (mm*)			
		4mg/ml	3mg/ml	2mg/ml	1mg/ml
Staphylococcus aureus	Methanol	18.0	16.5	13.5	12.0
	Ethanol	19.5	17.5	13.5	10.0
	Aqueous	16.5	15.0	13.5	12.5
Escherichia coli	Methanol	16.5	12.5	11.5	10.5
	Ethanol	20.0	18.0	17.0	13.5

	Aqueous	13.0	12.5	11.0	10.5
Pseudomonas aeruginosa	Methanol	18.0	16.5	12.5	12.0
	Ethanol	21.0	18.0	15.0	13.0
	Aqueous	14.0	12.5	12.0	11.0
Klebsiella pneumonia	Methanol	21.0	19.0	17.0	13.5
	Ethanol	20.0	19.0	18.0	12.5
	Aqueous	15.5	14.5	13.5	12.5
Lactobacillus	Methanol	16.5	13.5	12.5	10.5
	Ethanol	15.5	15.0	12.2	11.5
	Aqueous	14.0	13.5	11.5	10.5

Key: mm = millimeters, mg/ml = milligram per millimeter, *C. nitida = Cola nitida*
* Result significant @ P, 0.05

Table 5: Sensitivity patterns of Zones of Inhibition exhibited by the crude methanol, ethanol and aqueous extract of *C. acuminata* on some pathogens at different concentrations.

Bacteria	**Solvent**	**Zones of Inhibition (mm*)**			
		4mg/ml	**3mg/ml**	**2mg/ml**	**1mg/ml**
Staphylococcus aureus	Methanol	19.5	18.5	13.0	12.5
	Ethanol	20.0	18.5	13.5	12.0
	Aqueous	13.5	12.0	11.5	10.5
Escherichia coli	Methanol	21.5	19.5	15.5	13.5
	Ethanol	19.0	18.0	14.5	14.0
	Aqueous	16.5	13.5	12.0	10.5
Pseudomonas aeruginosa	Methanol	18.0	15.0	14.5	13.0
	Ethanol	20.0	18.5	16.5	12.0
	Aqueous	13.5	12.5	12.0	11.0
Klebsiella pneumonia	Methanol	19.0	17.5	14.0	10.5
	Ethanol	18.0	17.0	14.5	11.5
	Aqueous	15.0	13.5	12.5	11.5
Lactobacillus	Methanol	22.5	19.5	13.0	12.5
	Ethanol	26.5	22.5	18.5	
	Aqueous	12.5	11.5	11.0	10.5

Key: mm = millimeters, mg/ml = milligram per millimeter, *C. acuminata = Cola acuminata*
* Result significant @ *P*, 0.05

5. Conclusion

Results from the current study has shown the presence of phytochemicals in *Cola spp* which exhibited promising antimicrobial activity against a broad spectrum of clinical isolates. Another striking finding arising from this study is that all the extracts of *kola* seeds showed varying degrees of antimicrobial activity on the microorganisms tested, with the methanol extract demonstrating the best activity (bigger zones) against all the test organisms at all concentrations. This therefore reaffirms the ethno-pharmacological importance of kola seeds and a further confirmation of the pharmacological basis for its exploration in folklore medicine for the treatment of infectious diseases. Further collaborative study in this area intend to focus on the isolation and spectroscopic characterization of the bioactive ingredients in *Cola spp* which may serve as novel compounds for development of new and more effective antimicrobial therapies. This would prove very useful especially in this era where drug resistance is a major challenge. Apart from performing synergistic studies to evaluate the performance of these species of kola grown in South West, Nigeria when combined with orthodox medicine, there is also the need for toxicity tests to be performed on these extracts which in our view is a prelude to initiating clinical trials in subsequent drug development.

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