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Free radical scavenging potential and antibacterial activity of *Cola nitida and Garcinia kola* extracts against bacterial strains isolated from patients with urinary tract infections

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Abstract. *Cola nitida* and *Garcinia kola* are found and widely consumed in West Africa. The seeds of these plants have various traditional uses and are reported to exhibit several bioactivities. Their phytochemical, antioxidant and antibacterial properties of methanol, ethanol and aqueous extracts were investigated in the present study. Phytochemical screening and quantification of total phenolic contents analysis were carried out for phytochemical investigation. Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, saponins, protein and glycosides in the seed extracts. Quantitative phytochemical constituents revealed 0.818 ± 0.021and 0.700 ± 0.017mg of phenolic compounds and total flavonoid content of 25.63 ± 1.60 and 25.10 ± 1.85mg in *G. kola* and *C. nitida* respectively. The extracts showed potent antioxidant activities compared to standard antioxidants by significantly inhibiting 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radical (·OH), and superoxide anion radicals (O_2·) dose dependently. The methanol extracts of *G. kola* and *C. nitida* showed significant inhibitory action ($p<0.05$) against the bacterial isolates. The minimum inhibitory concentration obtained for methanol extract of the plants and both the mixture was 12mm at 31.25mg/ml for *Klebsiella pneumoniae* while the ethanol and aqueous extract of the plants and both the mixture was 13mm and 12.33mm at 31.25mg/ml and 125mg/ml respectively for *E. coli.* A direct correlation was observed between total phenolic content of extracts and radical scavenging potential, thus linking the observed bioactivities of these extracts to the presence of the phytochemical. The mixture of these seed extracts showed greater effect against the bacterial isolates, therefore providing a platform for advance studies in the development of drugs against infectious diseases.

Key words: Agar well dilution, antibacterial activity, *Cola nitida***,** *Garcinia kola***,** Free radical scavenging potential

1 Introduction

The alarming rate of antimicrobial drug resistance by pathogenic microorganisms against synthetic antibiotics (Maiyo *et al.* 2010) is a serious global problem. Indeed, the emergence of bacterial resistance to antibacterial drug today has become a common

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phenomenon, and consequently, antibiotic resistance has imposed both a biological and economic cost (Chabot *et al.* 2002, Chen *et al*. 2002, Chessin *et al*. 2005). The rate of antimicrobial resistance has prompted the search for new plants with antimicrobial properties and potentials to serve as sources of raw material for the synthesis of new drugs (Akoachere *et al*. 2002).

Traditional healers use different plant medicines to provide health care to most of the people in a curative rather than a preventive approach in the developing countries for common ailments (Gabriel *et al.* 2007). The availability and economy of these plants as direct therapeutic agents makes it more attractive when compared to modern medicine (Agbo and Ngogang 2005, Agbo *et al.* 2005). Natural plants contain phytochemical properties similar to synthetic antibiotics and have been used in folk medicine to treat infections (Ezeigbo 2016). In addition, people with different cultural backgrounds from ancient times to the present day have used herbal medicines (El-Mahmood *et al*. 2008) to cure infections. Hence, plants continue to be the most preferred exclusive source of drugs for the majority of the world's population (Fabiola *et al*. 2003, Jonathan and Fasidi 2003, Ajayi *et al*. 2008). According to WHO (2000), "medicinal plants when administered to man or animals exert a sort of pharmacological action on them". For this reason, medicinal plants are used as sources to produce useful drugs utilized by people worldwide for treatment of infectious diseases.

Infectious diseases are the major causes of death accounting for approximately one half of all deaths in tropical countries (Iwu *et al*. 2009). In recent times, medicinal plants continue to play a major role in primary healthcare as therapeutic remedies in many developing countries (Jonathan and Fasidi 2003, 2005, Jonathan *et al*. 2007) as some plants have been found to be rich in secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids and volatile oil. These compounds are said to be responsible for their therapeutic activities (Rabe and Vanstoden 2000, Cowan 2009). Furthermore, plants can serve as a reservoir of effective chemotherapeutic agent which provides valuable natural drug for effective and efficient management of human and plant diseases (Kanomal *et al*. 2014).

In Nigeria, studies have been carried out on a variety of these medicinal plants yet a good number of them with putative medicinal and antimicrobial potentials are yet to be studied (Amalu *et al.* 2014). Among these plants are *Garcinia kola* and *Cola nitida* whose medicinal uses may have not been fully explored in the treatment of bacterial infections, especially, urinary tract infections. These medicinal properties could occur in different forms varying from biological, synthetic chemotherapeutic, antibiotics, and phytotherapeutic agents (Arekemase *et al*. 2012). The action of these agents could either be 'bactericidal' or 'bacteriostatic' (Arekemase *et al*. 2012). The importance and quest for these medicinal plants origin that could be of potential benefit as antibacterial agents stimulated the interest in *Garcinia kola* ('bitter kola') and *cola nitida* (kola nut) seeds which are widely consumed as stimulant (Atawodi *et al*. 2005).

Garcinia kola, also generally known as 'Bitter kola' is a flowering plant species that belongs to the family of tropical plants known as Guttiferae or Clusiaceae (Adesuyi *et al*. 2012). In Nigerian languages, it is commonly called "Namijin Goro" in Hausa, "Orogbo" in Yoruba, and "Agbilu" in Igbo (Dalziel 2008). Bitter kola is also

known as African wonder nut because almost every part of it has been found to be of medicinal importance (Adegboye *et al*. 2008).

Cola nitida (Kola nut) ("Goro" in Hausa; "Obi gbanja" in Yoruba; "Oji" in Igbo, Keay *et al*. 2014) is a member of the family Steculicca. It is a tree plant found in Sierra Leone, North Ashanti, tropical Western Africa, West Indies, Brazil and Java (Grieve, 2001). *Cola nitida* was originally distributed along the west coast of Africa from Sierra Leone to the Republic of Benin with the highest frequency and variability occurring in the forest areas of Côte d'Ivoire and Ghana (Opeke 2012). In addition, kola nut is a native stimulant which commonly chewed in many West African cultures (Opeke 2012). In Nigeria, it is often used in traditional occasions, to welcome guest and receive visitors at home.

More so, the need for new antimicrobial agents is closely related with the problem of emergence of resistant strains to most antibiotics. Hence, this study was conducted to determine the phytochemical constituents, free radical scavenging potential and antibacterial activity of *Cola nitida and Garcinia kola*.

2 Materials and Methods

2.1 Isolation and Identification

Sixty urine samples were collected one each from female patients attending Federal Medical Centre, Yenagoa, from the period of May to July in 2017. Females were used because they were the available patients at the time of the study and are considered to be more predisposed to urinary tract infections. The specimens were cultured on MacConkey agar, Blood agar and CLED (Cystine lactose electrolyte deficient) agar plates using the streak method. Different agar was used to selectively identify and differentiate the possible bacteria including the fastidious organism that might be present in the culture specimen. Plates were inoculated and incubated at 37^{o}C for 24 hours. The isolates were identified using Gram staining technique and Biochemical tests which include catalase, urease, coagulase, oxidase, and indole.

2.2 Collection and authentication of plant material

Dried seeds of *Garcinia kola* (bitter kola) and *Cola nitida* (kola nut) were procured from a local herb dealer at Swali market in Yenagoa Local Government Area, Bayelsa State, Nigeria. They were authenticated with voucher specimen number MP-182 and MP-183 in the Pharmacognosy Department, Madonna University, Nigeria, Elele, Rivers State, Nigeria.

2.3 Processing and extraction

The seeds of *Garcinia kola* and *Cola nitida* were peeled, thoroughly washed and rinsed in distilled water, and both were sliced into tiny pieces with the use of a clean stainless

steel knife, then air-dried at room temperature for 4 weeks, and pulverized using laboratory mortar and pestle. Different organic solvents (methanol, ethanol and aqueous) were used for the extraction of these plants as described by Alade and Irobi (1993). Fifty grams (50 g) of each seed powder was dispensed into a cotton wool stock thimble chamber of the soxhlet apparatus and 500 ml of methanol was dispensed into flat bottom flask. The extraction solvents were heated in the bottom flask, vaporized into the thimble, condensed in the condenser and dripped back. When the liquid content reached the siphon arm, the liquid contents was emptied into the bottom flask again and the process was continued until the absorbent was clear. The extracts obtained were completely evaporated (Green 2004) and stored in the refrigerator at 4^{o}C until use. The percentage (%) yields of the dry residue were calculated (Pudhom *et al*. 2007). The same procedure was repeated successfully for ethanol and aqueous extracts. Extracts were then dissolved in the appropriate solvent for the phytochemical and antibacterial assay.

2.4 Phytochemical screening

Phytochemical screening was done using qualitative and quantitative phytochemical analysis. Qualitative analysis involved tests for flavonoids, tannins, carbohydrates, glycosides, saponins, resins, terpenoids and alkaloids. These were carried out using standard methods (Harborne 1984, Sofowora 1993, Trease and Evans 2001).

Quantitative Analysis determined the total phenols, tannin, total flavonoids and total anthocyanin contents. The total phenolics were determined using Folin-Ciocalteau reagent (FCR) as described by Velioglu *et al.* (1998) with slight modifications. Tannin content in each sample was determined using insoluble polyvinyl-polypirrolidone (PVPP), which binds tannins as described by Makkar *et al*. (1993). The flavonoids content was determined according to the method described by Kumaran and Karunakaran (2006) with slight modifications. This method was based on the formation of a flavonoid-aluminum complex, which absorbs maximally at 415 nm. The total anthocyanin contents of the plant extracts were measured using a spectrophotometric pH differential protocol described by Giusti and Wrolstad (2001) and Wolfe *et al.* (2003) with slight modifications.

2.5 *In vitro* Antioxidant Assays

Quantitative DPPH radical-scavenging assay

The hydrogen atoms or electrons donation ability of the corresponding extract was measured from the bleaching of purple coloured methanol solution of DPPH. The scavenging activity on DPPH free radicals by the extract was assessed according to the method reported by Gyamfi *et al.* (1999) with slight modifications.

Hydroxyl radical (·OH)-scavenging assay

The 2-deoxyribose assay was used to determine the scavenging effect of the extract on the hydroxyl (·OH) radical, as reported by Halliwell *et al.* (1987) with minor modifications.

Superoxide Radical ($O_2^{\cdot-}$) Scavenging Assay

This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) (Beauchamp and Fridovich 1971) and the method used by Martinez *et al.* (2001) to determine superoxide dismutase with slight modifications.

2.6 Reconstitution of plant extract

For preliminary screening of antibacterial activity of the plant extracts against bacteria isolated, all the dried extracts were dissolved in dimethyl sulfoxide (DMSO) to a final stock concentration of 2.5% w/v. As DMSO has been shown not to have any inhibitory effect on the growth of microorganisms (Zgoda and Porter 2001, Kuete *et al.* 2008), it was used as the negative control for all the experiments. A two-fold serial dilution was also undertaken to obtain lower concentration ranges in sterile test tubes.

2.7 Antibacterial Aassay

Preparation of 0.5 Mcfarland turbidity standards was done as described in NCCLS (NCCLS 2010). The agar well diffusion method was done following Atata (2003). An overnight agar-culture of each bacterial isolate was made, and the suspension of microorganisms was made in sterile normal saline and adjusted to 0.5 McFarland standards (10^8 CFU/ml) (NCCLS 2010). From the stock of 500 mg/ml extract, two-fold serial dilutions were made to 250, 125, 62.5, and 31.25 mg/ml. Each labeled Mueller Hinton agar plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth could be observed. A sterile cork borer of 6mm diameter was used to make 5 wells on the medium in each plate. Before boring of the well in agar, the cork borer was sterilized by dipping in alcohol and flaming. 50 µl of the 5 different extract concentrations were dropped into each well using a micropipette. All antibacterial assays were performed on duplicate plates. The underside of each well was appropriately labeled. Other solvents used for extraction apart from water were tested for each organism. The inoculated plates were kept in the refrigerator for 1 hour to allow the extracts to diffuse into the agar (Atata *et al*. 2003). The plates were incubated upright at 37°C for 24 hours. After incubation, the diameters of the zones of inhibitions obtained were measured, using a pair of calipers and meter ruler. The measurement was done at the back of the plate. The diameter was measured from one end of the zone to the other. Where the zone of inhibition is not perfectly circular, the average of the long and short axis was used. The diameter of the zone of inhibition was obtained for

the two plates having the same concentration of the extract against a particular micro-organism, and the average was used. For positive control, 30µl of 40 mg/ml of gentamycin was used while 50 µl of 2.5% DMSO was used as negative control.

2.8 Determination of minimum inhibitory concentration

The minimal inhibitory concentrations (MICs) of the extracts on the bacterial isolates were determined by macro broth dilution techniques following the recommendation of the Clinical and Laboratory Standard Institute (CLSI 2015).

One gram of the extract was dissolved in 1 ml of 20% DMSO to get an extract concentration of 250 mg/ml. Various serial dilutions were made from this stock solution in tubes of 1 ml sterile Mueller Hinton broths to get 125 mg/ml, 62.5 mg/ml, and 31.25 mg/ml. An overnight nutrient broth culture of the test bacterial isolate was standardized to 0.5 McFarland turbidity standards. Different dilutions of the suspension were made in a sterile normal saline to obtain a final inoculum concentration of 106 CFU/ml. Then 1 ml of this adjusted inoculum was added to each tube of the Mueller Hinton broth containing different concentration of the crude extract. Each tube was mixed and incubated at 37^{o}C for 24 hours (Nweze and Onyishi 2010). This experiment was conducted in duplicate for all the bacterial isolates. A tube of Mueller Hinton broth containing only the 1ml suspension of the isolate without extract and the tubes of Mueller Hinton broth containing different concentrations of the extract without the isolate were used as controls. The tubes were examined after 24 hrs incubation. The MIC of the extract was taken as the lowest extract concentration that completely inhibited the growth of the bacterial isolates in the tubes, as indicated by lack of visual turbidity.

2.9 Determination of the use of mixtures of the extract

This was done by measuring equal volume of each of extract type (ethanol, methanol and aqueous) of the plants seeds and then mixed. 50µl of each mixture was put into each well as in the antibacterial bioassay section to test the sensitivity potentials. This was done in triplicates.

2.10 Statistical analysis

All experiments were done in triplicate and the data thus obtained were reported as mean ± standard error of mean. Statistical analysis was carried out to determine whether there was significant difference among the inhibitory actions of *Garcinia kola* and *Cola nitida* alone and in mixtures of extracts using Analysis of Variance and Bonferroni post-test at 95% confidence level using Graph Pad PRISM Version 5.01 (Chao-Hsun *et al.* 2010).

3 Results

Total of 48 isolates were obtained from the 60 specimens collected from patients with urinary tract infections (UTI) attending Federal Medical Centre. Bacterial species isolated include *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Table 1 shows the frequency distribution of bacterial isolates from patients with UTI. *Escherichia coli* had the highest occurrence (29.17%) while *Proteus vulgaris* had the lowest occurrence (8.33%).

Table 1: Frequency distribution of bacterial isolates (48) from patients with urinary tract infections.

Organisms	Number of isolates	Percentage occurrence
Proteus vulgaris	4	8.33
Escherichia coli	14	29.17
Staphylococcus aureus	12	25.00
Pseudomonas aeruginosa	8	16.67
Klebsiella pneumoniae	10	20.83

Table 2 shows the zone of inhibition of the positive control (gentamycin) used against bacterial isolates. The zones of inhibition produced by the positive control were larger than the zones produced by the plant extracts.

Table 2: Zones of Inhibition (mm) of Gentamycin (positive control) against bacterial isolates.

Organisms	Concentration (µl)	Zone of inhibition (mm) Gentamycin [a]
Proteus vulgaris	30.0	28.00
Escherichia coli	30.0	26.00
Staphylococcus aureus	30.0	18.00
Pseudomonas aeruginosa	30.0	26.00
Klebsiella pneumoniae	30.0	29.00

[a] Values are mean inhibition zone (mm) from three replicates

The methanol extracts of *G. kola*, *C. nitida* and the mixture of extracts showed antibacterial activity against all the bacterial isolates at a concentration of 500 mg/ml, with *G. kola* having the largest zone of inhibition of 23 mm against *E. coli* and *Klebsiella pneumoniae* respectively. *C. nitida* showed activity against *Klebsiella pneumoniae* with largest zone of inhibition of 21.33 mm while the mixture of both extracts showed activity against *P. vulgaris* and *E. coli* with the zone of inhibition of 25.66 mm respectively ($p < 0.05$) as shown in Table 3.

Table 3: Mean zone of Inhibition (mm) of Methanol extract of *Garcinia kola*, *Cola nitida* and mixture of extracts against bacterial isolates.

Bacterial isolate	Plant extract	Concentration				
		500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.5 mg/ml
Proteus vulgaris	*Garcinia kola*	21.33 ± 0.88^{A}	20.00 ± 1.15^{D}	17.33 ± 0.67^{G}	13.00 ± 0.58^{J}	NI
	Cola nitida	17.33 ± 0.33^{B}	16.00 ± 0.58^{E}	13.66 ± 0.33^{H}	11.33 ± 0.33^{J}	NI
	Mixture of extracts	25.66 ± 0.33^{C}	22.33 ± 0.33^{F}	20.00 ± 0.58^{I}	16.66 ± 0.33^{K}	14.33 ± 0.33^{L}
Escherichia coli	*Garcinia kola*	23.00 ± 0.58^{A}	21.66 ± 0.88^{D}	19.66 ± 0.33^{G}	17.00 ± 0.58^{J}	14.66 ± 0.33^{M}
	Cola nitida	19.00 ± 0.58^{B}	16.00 ± 0.58^{E}	14.00 ± 0.58^{H}	12.33 ± 1.20^{K}	0.00 ± 0.00^{N}
	Mixture of extracts	25.66 ± 0.33^{C}	21.33 ± 0.33^{D}	18.33 ± 0.33^{G}	15.00 ± 0.58^{J}	13.33 ± 0.33^{M}
Staphylococcus aureus	*Garcinia kola*	17.66 ± 0.33^{A}	16.00 ± 0.58^{CD}	14.66 ± 1.20^{E}	NI	NI
	Cola nitida	17.66 ± 0.88^{A}	15.66 ± 0.33^{C}	0.00 ± 0.00^{F}	NI	NI
	Mixture of extracts	20.00 ± 0.58^{B}	17.66 ± 0.33^{D}	15.66 ± 0.33^{E}	NI	NI
Pseudomonas aeruginosa	*Garcinia kola*	15.66 ± 0.33^{A}	13.00 ± 0.58^{C}	0.00 ± 0.00^{E}	NI	NI
	Cola nitida	16.33 ± 0.67^{A}	13.66 ± 0.33^{C}	0.00 ± 0.00^{E}	NI	NI
	Mixture of extracts	19.00 ± 0.58^{B}	16.00 ± 0.58^{D}	10.33 ± 0.58^{F}	NI	NI
Klebsiella pneumoniae	*Garcinia kola*	23.00 ± 0.58^{AB}	20.33 ± 0.33^{C}	18.33 ± 0.67^{E}	14.00 ± 0.58^{G}	0.00 ± 0.00^{H}
	Cola nitida	21.33 ± 0.33^{A}	20.00 ± 0.58^{C}	18.66 ± 0.33^{E}	14.00 ± 0.58^{G}	0.00 ± 0.00^{H}
	Mixture of extracts	25.33 ± 0.33^{AB}	22.00 ± 1.15^{C}	20.33 ± 1.20^{E}	16.33 ± 0.33^{G}	12.00 ± 0.58^{I}

Values are given as Mean ± Standard Error of three replicate plates
NI = No inhibition
All values of a particular isolate within the same column with shared alphabet superscript are non-significant (P>0.05).

Table 4: Mean zone of Inhibition (mm) of Ethanol extract of *Garcinia kola*, *Cola nitida* and mixture of extracts against bacterial isolates.

Bacterial isolate	Plant extract	Concentration				
		500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.5 mg/ml
Proteus vulgaris	*Garcinia kola*	20.00 ± 0.58^{A}	18.00 ± 0.58^{D}	15.00 ± 0.58^{G}	13.66 ± 0.33^{J}	NI
	Cola nitida	16.00 ± 0.58^{B}	14.33 ± 0.33^{E}	12.00 ± 0.58^{H}	0.00 ± 0.00^{K}	NI
	Mixture of extracts	25.33 ± 0.33^{C}	22.33 ± 0.33^{F}	18.33 ± 0.88^{I}	15.33 ± 0.88^{J}	NI
Escherichia coli	*Garcinia kola*	22.66 ± 0.67^{A}	20.66 ± 0.67^{DF}	18.66 ± 0.33^{GI}	16.66 ± 0.88^{JL}	15.66 ± 0.33^{M}
	Cola nitida	18.66 ± 0.67^{B}	16.00 ± 0.58^{E}	14.33 ± 0.33^{H}	0.00 ± 0.00^{K}	0.00 ± 0.00^{N}
	Mixture of extracts	25.66 ± 0.58^{C}	21.33 ± 0.67^{F}	17.33 ± 0.33^{I}	15.00 ± 0.58^{L}	13.00 ± 0.58^{O}
Staphylococcus aureus	*Garcinia kola*	16.50 ± 0.33^{A}	13.66 ± 0.33^{C}	0.00 ± 0.00^{E}	NI	NI
	Cola nitida	17.00 ± 0.58^{AB}	15.00 ± 0.58^{CD}	13.33 ± 0.33^{F}	NI	NI
	Mixture of extracts	18.00 ± 0.58^{B}	15.66 ± 0.33^{D}	13.66 ± 0.67^{F}	NI	NI
Pseudomonas aeruginosa	*Garcinia kola*	15.66 ± 0.33^{A}	12.66 ± 0.33^{D}	0.00 ± 0.00^{F}	NI	NI
	Cola nitida	14.50 ± 0.33^{A}	13.83 ± 1.00^{DE}	0.00 ± 0.00^{F}	NI	NI
	Mixture of extracts	17.33 ± 0.33^{B}	15.00 ± 0.58^{E}	12.66 ± 0.33^{G}	NI	NI
Klebsiella pneumoniae	*Garcinia kola*	21.33 ± 0.67^{AB}	17.33 ± 0.33^{C}	15.00 ± 0.58^{E}	0.00 ± 0.00^{G}	NI
	Cola nitida	19.66 ± 0.33^{A}	16.66 ± 0.88^{C}	14.00 ± 0.58^{E}	0.00 ± 0.00^{G}	NI
	Mixture of extracts	22.33 ± 0.33^{B}	19.66 ± 0.88^{D}	17.66 ± 0.33^{F}	12.33 ± 0.33^{H}	NI

Values are given as Mean ± Standard Error of three replicate plates; NI = No inhibition
All values of a particular isolate within the same column with shared alphabet superscript are non-significant (P>0.05).

Ethanol extracts of *G. kola, C. nitida* and the mixture of extracts showed antibacterial activity against all the bacterial isolates at a concentration of 500 mg/ml. *G. kola* inhibited the growth of *E. coli* with 22.66 mm as the largest zone of inhibition while *C. nitida* showed its highest activity against *Klebsiella pneumoniae* at zone of inhibition of 19.6 mm. Furthermore, the mixture of extracts showed its effectiveness with the largest zone of inhibition of 25.66 mm against *E. coli* with significance of ($p< 0.05$) (Table 4).

Table 5 shows the aqueous extracts of *G. kola, C. nitida* and the mixture of extracts against the bacterial isolates. *G. kola, C. nitida* and the mixture of extracts showed antibacterial activity against all the bacterial isolates at a concentration of 500 mg/ml except against *Staphylococcus aureus*. The effect of *G. kola* was seen against *P. vulgaris* with the largest zone of inhibition of 17 mm while *C. nitida* was able to inhibit the growth of *E. coli* with the largest zone of inhibition of 16.66 mm. The mixture of both extracts equally showed effectiveness against *P. vulgaris* with the largest zone of inhibition of 20 mm which shows similar results with Omwirhiren *et al.* (2016).

Table 5: Mean zone of inhibition (mm) of aqueous extract of *Garcinia kola*, *Cola nitida* and mixture of extracts against bacterial isolates.

Bacterial isolate	Plant extract	Concentration*		
		500 mg/ml	250 mg/ml	125 mg/ml
Proteus vulgaris	*Garcinia kola*	17.00 ± 0.58^{A}	15.33 ± 0.33^{D}	12.66 ± 0.33^{G}
	Cola nitida	12.66 ± 0.88^{B}	10.66 ± 0.33^{E}	0.00 ± 0.00^{H}
	Mixture of extracts	20.00 ± 0.58^{C}	16.00 ± 0.58^{D}	13.33 ± 0.33^{G}
Escherichia coli	*Garcinia kola*	15.00 ± 0.58^{A}	13.66 ± 0.33^{C}	0.00 ± 0.00^{E}
	Cola nitida	16.66 ± 1.20^{A}	14.66 ± 0.33^{C}	12.33 ± 0.33^{F}
	Mixture of extracts	19.00 ± 1.00^{B}	14.33 ± 0.33^{C}	12.66 ± 0.33^{F}
Staphylococcus aureus	*Garcinia kola*	NI	NI	NI
	Cola nitida	NI	NI	NI
	Mixture of extracts	NI	NI	NI
Pseudomonas aeruginosa	*Garcinia kola*	12.33 ± 0.88^{A}	NI	NI
	Cola nitida	0.00 ± 0.00^{B}	NI	NI
	Mixture of extracts	13.00 ± 0.58^{A}	NI	NI
Klebsiella pneumoniae	*Garcinia kola*	15.33 ± 0.67^{A}	13.00 ± 0.58^{D}	NI
	Cola nitida	0.00 ± 0.00^{B}	0.00 ± 0.00^{E}	NI
	Mixture of extracts	13.33 ± 0.33^{C}	11.66 ± 0.33^{F}	NI

* There was no inhibition detected against any of the bacterial isolates at 62.5 mg/ml 31.5 mg/ml concentrations of the aqueous extracts of single plant species or both plants in mixture, so that those two columns were not shown in the table.

Values are given as Mean ± Standard Error of three replicate plates; NI = No inhibition;

All values of a particular isolate within the same column with shared alphabet superscript are non-significant ($P>0.05$).

Table 6 shows the Minimum Inhibitory Concentration (MIC) values for the extracts of *G. kola, C. nitida* and the mixture of extracts against bacteria isolated from UTI. The

MIC values of the extracts against the isolates were obtained from the agar diffusion assay. The lowest MIC for *G. kola*, *C. nitida* and the mixture of extracts were obtained with methanol and ethanol extracts against all bacterial species respectively. However, there was no MIC obtained for *S. aureus*, *P. aeruginosa*, and *K. pneumonia* for aqueous extract, and no MIC obtained for *S. aureus* for aqueous mixture of extracts as well.

Table 6: Minimum Inhibitory Concentration (MIC) values of extract of *G. kola*, *C. nitida* and mixture of extracts against bacterial isolates.

Isolate	Plant	Minimum Inhibitory Concentration (mg/ml)		
		Methanol	Ethanol	Aqueous
Proteus vulgaris	*Garcinia kola*	62.5	62.5	125
	Cola nitida	62.5	125	250
	Mixture of extracts	31.25	62.5	125
Escherichia coli	*Garcinia kola*	31.25	31.25	250
	Cola nitida	62.5	125	125
	Mixture of extracts	31.25	31.25	125
Staphylococcus aureus	*Garcinia kola*	62.5	250	250
	Cola nitida	62.5	125	No MIC
	Mixture of extracts	62.5	125	250
Pseudomonas aeruginosa	*Garcinia kola*	250	125	500
	Cola nitida	125	125	No MIC
	Mixture of extracts	125	62.5	500
Klebsiella pneumoniae	*Garcinia kola*	62.5	62.5	250
	Cola nitida	31.25	62.5	No MIC
	Mixture of extracts	31.25	31.25	250

Qualitative analysis on *Garcinia kola* and *Cola nitida* revealed the presence of important phytochemical constituents including phenolic compounds (tannins and flavonoids), saponins and alkaloids as bioactive compounds (Table 7).

Table 7. Qualitative phytochemical constituents of *Garcinia kola* and *Cola nitida extracts.*

Extract	Flavonoids	Tannins	Alkaloids	Terpenoid	Glycoside	Saponins	Resin	Protein
G. kola	+	+	+ +	-	+	+ +	-	+
C. nitida	+ +	+ +	+	-	+	+	-	+

(Key: + Present; + + Moderately present; + + + Abundant; - Absent)

Quantitative analysis on *Garcinia kola* and *Cola nitida* revealed that phenolic compounds were a major class of bioactive components in the extracts Table 8). The amount of total phenolics were 0.818 ± 0.021 mg and 0.700 ± 0.017 mg GAE/mg of dry plant extracts of *G. kola* and *C. nitida* respectively, whereas the total flavonoid contents were as 25.63 ± 1.60 mg and 25.10 ± 1.85 rutin equivalents / g dry weight plant extract of *G. kola* and *C. nitida* respectively.

Table 8: Phytochemical constituents of *Garcinia kola* and *Cola nititda* (mean ± SD, n=3).

Extract	Phenolic contents *			† Total anthocyanin	‡ Total flavonols	‡ Total flavonoids
	Total Phenols	Non-tannins	Tannins			
G. kola	0.818 ± 0.021	0.507 ± 0.009	0.311 ± 0.001	4.64 ± 0.22	9.41 ± 0.02	25.63 ± 1.60
C. nitida	0.700 ± 0.017	0.376 ± 0.001	0.324 ± 0.007	2.15 ± 0.22	13.81 ± 0.92	25.10 ± 1.85

* Expressed as mg gallic acid equivalents / mg dry weight plant extract
† Expressed as mg cyanidin 3-glucoside equivalents/100g of dry weight extract
‡ Expressed as mg rutin equivalents / g dry weight plant extract

Table 9 shows the concentration of the extracts that inhibited 50% of the free radicals and lipid peroxidation (IC_{50}) which was used to determine the potency of the extracts. The lower the IC_{50} value the better the extract potency. The plant extracts were efficient inhibitors of different free radicals compared to standard antioxidants. *G. kola* appears to be more efficient in inhibiting DPPH radical (9.6 ± 1.0 μg/ml), Superoxide anion (64.6 ± 1.5 μg/ml) and lipid peroxidation (282.9 ± 9.3 μg/ml) while *C. nitida* extract is a better inhibitor of Hydroxyl radical (46.6 ± 2.5 μg/ml).

Table 9: Free radical and lipid peroxidation inhibitory potency (IC_{50}) of *Garcinia kola* and *Cola nititda* (mean ± SEM, n=3).

Extract	IC_{50} value for inhibitory potential (μg/ml)			
	DPPH radical	Hydroxyl radical (·OH)	Superoxide anion ($O_2^{\cdot-}$)	Lipid peroxidation
C. nitida	24.1 ± 2.1	46.6 ± 2.5	103.7 ± 5.2	575.1 ± 15.4
G. kola	9.6 ± 1.0	99.4 ± 1.7	64.6 ± 1.5	282.9 ± 9.3
Standard antioxidant	4.1 ± 0.3 *	38.9 ± 2.8 #	3.3 ± 0.2 β	24.3 ± 1.4 £

* compared to ascorbic acid; # compared to α-Tocopherol; β compared to rutin;
£ compared to butylated hydroxyltoluene

Figure 1 shows the graphical representation of *Garcinia kola* and *Cola nitida* extracts which showed significant dose-dependent DPPH radical scavenging capacity. *Garcinia kola* appears to be more efficient, inhibiting 92.36 ± 1.31% of DPPH at a concentration of 125 μg/ml compared to ascorbic acid which inhibited 94.18 ± 3.22 % at the same concentration.

Figure 2 shows the graphical representation of *Garcinia kola* and *Cola nitida* extracts scavenged ·OH radical in a concentration dependent manner. The two extracts inhibited 2-deoxyribose degradation above 30% with maximal inhibition of 76.7 ± 1.4% at concentration of 500 μg/ml by *S. s*. The scavenging ability of the extracts was significant at all tested concentrations. *C. nitida* extract was found to be powerful quencher of ·OH radical thereby preventing the propagation of lipid peroxidation. At high concentrations of both extracts lower activities were observed.

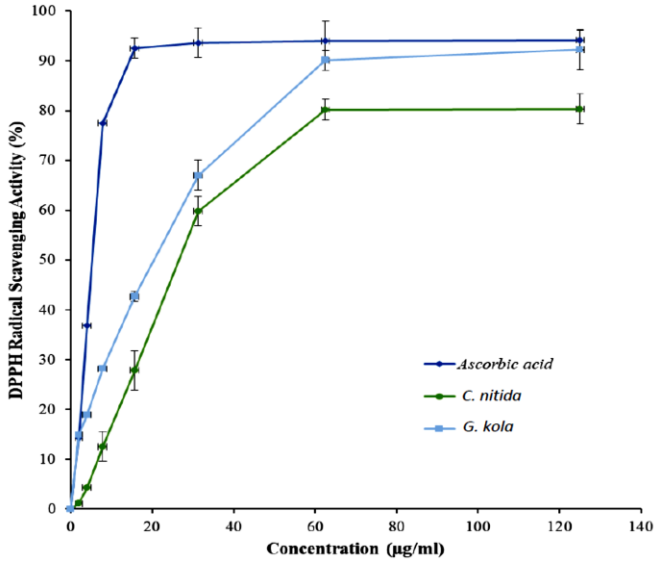

Fig. 1: Graph comparing DPPH antioxidant activity of different concentrations of ascorbic acid and extracts of *Garcinia kola* and *Cola nitida* (values are expressed as mean ± SEM, n = 3)

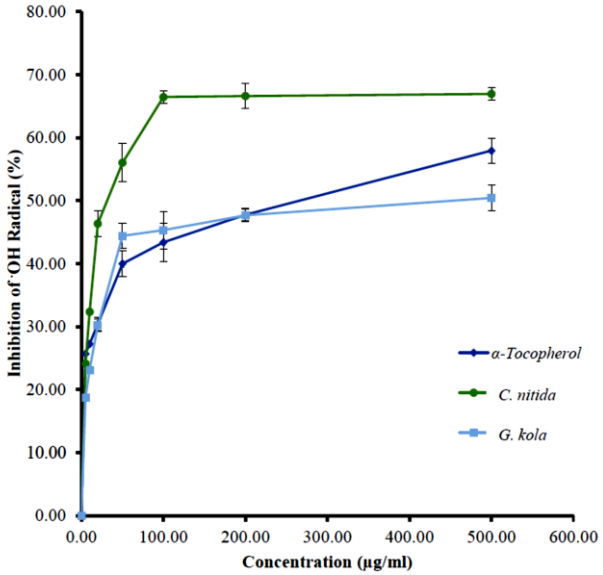

Fig. 2: Graph comparing Hydroxyl (·OH) antioxidant activity of different concentrations of α-tocopherol and extracts of *Garcinia kola* and *Cola nitida*. Values are expressed as mean ± SEM, n = 3)

Figure 3 shows the *Garcinia kola* and *Cola nitida* extracts which inhibited the formation of reduced NBT in a dose-related manner. *C. nitida* showed the maximal

$O_2^{\cdot-}$ anion inhibitory activity of 86.68±2.10% at the concentration of 250 µg/ml, compared to rutin (96.03±2.2%, at 250 µg/ml). The $O_2^{\cdot-}$ scavenging effect of the extracts could culminate in the prevention of $^{\cdot}OH$ radical formation since $O_2^{\cdot-}$ and H_2O_2 are required for $^{\cdot}OH$ radical generation.

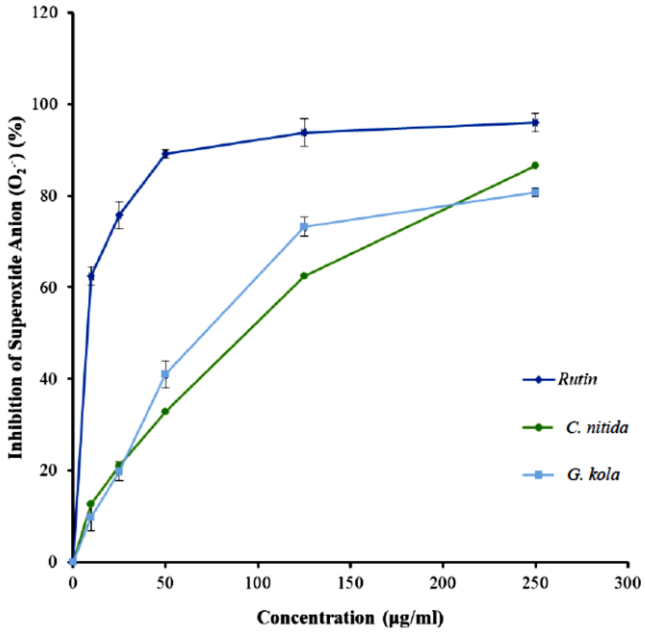

Fig. 3: Graph comparing superoxide anion ($O_2^{\cdot-}$) inhibition of different concentrations of rutin and extracts of *Garcinia kola* and *Cola nitida* (values are mean ± SEM, n = 3)

4 Discussion

The involvement of bacteria in urinary tract infection is of great concern. Female patients were only participant involved in this study because they are considered to be more predisposed to urinary tract infections. Studies have shown *Escherichia coli* and *Staphylococcus saprophyticus* as mostly implicated in urinary tract infection (Nicolle 2008). However, this study revealed the presence of *Escherichia coli* as the highest occurring bacteria with 14 (29.17%), while *Proteus vulgaris* as the lowest with 4 (8.33%) which agrees with Mansour *et al.* (2009). The presence of these bacteria could possibly be because of poor sanitary hygiene.

The findings from this study revealed the presence of alkaloids, saponins, tannins, flavonoids, glycoside, protein and the absent of terpenoids and resin in the extracts of *Garcinia kola* and *Cola nitida* in the methanol, ethanol and aqueous used as solvent as agreed with work done on *C. nitida and G. kola* by Omwirhiren *et al.* (2016). Studies have shown that the various solvents influence the nature of compounds extracted and their bioactivities (Arunkumar and Muthuselvam 2009, Seanego 2012). However, methanol extracts appear to be most potent and promising as shown by its high

inhibitory activity against the clinical isolates. This could be attributed to the high presence of some of the polyphenolic compound identified (total flavonoids content of 25.63 ± 1.60 and 25.10 ± 1.85 mg rutin equivalents/g dry weight of *G. kola* and *C. nitida* respectively). These results clearly show that the solvent influences the extractability of the phenolic compounds. The phenolic extracts of plants are always a mixture of different classes of phenols, which are selectively soluble in the solvents. This finding is in conformity with previous studies by Ukaoma *et al.* (2013) and Alaje *et al.* (2014). The presence of these secondary metabolites is known to have therapeutic activity against several diseases and therefore could suggest the basis for their traditional use for the treatment of various illness (Yousuf *et al*. 2012) including urinary tract infections. Earlier studies have reported that flavonoids have antibacterial property as they have the capability to associate with soluble proteins and bacterial cell walls (Doss *et al*. 2011).

The evaluation of the antibacterial properties and the effect of mixture of extracts on bacterial isolates showed that they all possess antibacterial properties. The antibacterial activity was seen at varying concentrations indicating that the plant extract had broad antibacterial spectrum (Bankole 1992). Presence of alkaloids and flavonoids in *G. kola* and *C. nitida* has been observed to be responsible for its antibacterial property. However, the data obtained showed that the inhibitory effects of these plant extracts on the various bacterial isolates were dose dependent. This observation agrees with the findings of Agbaje *et al.* (2006) and Akinnibosun *et al*. (2009). The methanol extract of *G. kola* was most active against *E. coli, K. pneumoniae, P. vulgaris, S. aureus,* and *P. aeruginosa* with zones of inhibition ranging from 23.00 mm to 13.00 mm. The ethanol extract of *G. kola* extract was active against *E. coli, K. pneumoniae, P. vulgaris, S. aureus,* and *P. aeruginosa* with zones of inhibition ranging from 22.66 mm to 12.66 mm. While the aqueous *G. kola* was active against *P. vulgaris, E. coli, K. pneumoniae, P. aeruginosa* with zones of inhibition ranging from 17.00 mm to 12.33 mm and showed no zone of inhibition to *S. aureus*. This result is similar to the work of Adegboye *et al.* (2008), who showed that the crude extract of *G. kola* exhibited antibacterial activities in vitro against both Gram-positive and Gram-negative organisms. The antibacterial properties of this plant could be attributed to the presence of tanins and flavonones. Studies have shown it to have good antibacterial, antifungal and antiviral properties (Terashima *et al.* 2002, Adesuyi *et al.* 2012). Other medicinal properties of the plant include its usage in the treatment of skin infections in Liberia and Congo Democratic Republic. The powdered bark of the plant is applied to malignant tumors and cancers, whereas the plants latex is taken internally for gonorrhea and externally to seal new wounds and prevent sepsis (Adesuyi *et al*. 2012). In Nigeria, a cold-water extract of the roots and bark with salt are administered to cases of bronchial asthma or cough and vomiting (Adesuyi *et al.* 2012).

Methanol extract of *C. nitida* was most active against *K. pneumonia, E. coli, S. aureus, P. vulgaris,* and *P. aeruginosa* with zones of inhibition ranging from 21.33 mm to 11.33 mm. Ethanol *C. nitida* extract was active against *K. pneumoniae, E. coli, S. aureus, P. vulgaris, P. aeruginosa* with zones of inhibition ranging from 19.66 mm to 12.00mm. While the aqueous *C. nitida* was active against *E. coli, P. vulgaris* with

zones of inhibition ranging from 16.66 mm to 10.66 mm and showed no zone of inhibition to *S. aureus, P. aeruginosa* and *K. pneumoniae.*

The mixture of the extracts produced greater zones of inhibition on the bacterial isolates than the zones of inhibition produced by *G. kola* and *C. nitida* when used separately. The effect of mixture of plant extracts on the bacterial isolates was seen with methanol extract and least with aqueous extract. The methanol mixture of extracts was observed to be most active against *P. vulgaris, E. coli, K. pneumoniae, S. aureus,* and *P. aeruginosa* with zones of inhibition ranging from 25.66 mm to 12.00 mm. The ethanol mixture of extracts was seen to be active against *E. coli, P. vulgaris, K. pneumoniae, S. aureus,* and *P. aeruginosa* with zones of inhibition ranging from 25.66 mm to 12.33 mm. While the aqueous mixture of both plants was also observed to be active against *P. vulgaris, E. coli, K. pneumoniae, P. aeruginosa* with zones of inhibition ranging from 20.00 mm to 11.66 mm and showed no zone of inhibition to *S. aureus*. With this result, the effect of the mixture of the extracts showed greater antibacterial activity against the bacterial isolates when favourably compared with the standard antibiotic. Although, no studies have shown this, that of *Solenostemon monostachyus* and *Ocimum gratissimum* (Chukwura and Iheukwumere, 2012) results showed they have greater inhibitory effect as *G. kola* and *C. nitida* extract mixture.

The MIC of the extracts against the bacteria was also determined varying between concentration of 31.25 mg/ml to 500 mg/ml for *G. kola* extract, *C. nitida* extract and the mixture of both extracts, respectively. The results of MIC showed that the mixture of extracts is more potent against the bacterial isolates even at low concentrations. The broad spectrum of activity displayed by the extracts in this study appears to justify and explain the basis for their uses in traditional medicine, possibly as a remedy to the emergence of drug-resistant strains caused by inappropriate use of orthodox antibiotics.

Garcinia kola and *Cola nitida* extracts showed significant dose-dependent DPPH radical scavenging capacity. *Garcinia kola* appears to be more efficient, inhibiting 92.36±1.31% of DPPH at a concentration of 125 µg/ml compared to ascorbic acid which inhibited 94.18 ± 3.22 % at the same concentration as proven by Okoko (2009). *Garcinia kola* and *Cola nitida* extracts scavenged ·OH radical in a concentration dependent manner. The two extracts inhibited 2-deoxyribose degradation above 30% with maximal inhibition of 76.7±1.4 % at concentration of 500 µg/ml. The scavenging ability of the extracts was significant at all tested concentrations. The high radical scavenging activity of *Garcinia kola* and *Cola nitida* seems to be directly correlated with its total phenolic content as it may play an important role in their antioxidative effect. *C. nitida* extract was also found to be powerful quencher of ·OH radical thereby preventing the propagation of lipid peroxidation. At high concentrations of both extracts lower activities were observed. *Garcinia kola* and *Cola nitida* extracts which inhibited the formation of reduced NBT in a dose-related manner. *C. nitida* showed the maximal $O_2^{\cdot -}$ anion inhibitory activity of 86.68±2.10% at the concentration of 250 µg/ml, compared to rutin (96.03 ± 2.2 %, at 250 µg/ml). The $O_2^{\cdot -}$ scavenging effect of the extracts could culminate in the prevention of ·OH radical formation since $O_2^{\cdot -}$ and H_2O_2 are required for ·OH radical generation. The observed ability of the extracts to scavenge or inhibit HO· radical indicated that the extracts could significantly inhibit

lipid peroxidation. This corroborates the studies of Farshori *et al*. (2013) and Olatunde *et al*. (2004) who reported that *G. kola* and *C. nitida* contains natural antioxidants.

The IC_{50} is the concentration of the extracts that inhibited 50% of the free radicals and lipid peroxidation which was used to determine the potency of the extracts. The lower the IC_{50} value the higher the extract potency. The plant extracts were efficient inhibitors of different free radicals compared to standard antioxidants. *G. kola* appears to be more efficient in inhibiting DPPH radical (9.6±1.0 µg/ml), Superoxide anion (64.6±1.5 µg/ml) and lipid peroxidation (282.9±9.3 µg/ml) while *C. nitida* extract is a better inhibitor of Hydroxyl radical (46.6±2.5 µg/ml). IC_{50} was calculated as the amount of antioxidant present in the sample necessary to decrease the initial DPPH concentration by 50%. The lower the IC_{50} value the higher is the antioxidant activity. The observed antibacterial property of these seeds could therefore be linked to the presence of the phenolic compounds as they have previously been found to be main contributors of antioxidant activity and are also responsible for anti-inflammatory, antiviral, anticancerous and antimicrobial activities (Yang *et al*. 2013).

5 Conclusions

The present study revealed the presence of phytochemicals in *G. kola* and *C. nitida* which exhibited promising antimicrobial activity against a broad spectrum of bacterial isolates. Another striking finding was that the extracts showed free radical scavenging potential properties against the synthetic oxidative molecules and varying degrees of antibacterial activity on the bacteria isolated, with the methanol extract demonstrating the most effective activity against all the isolate at all concentrations. This therefore reaffirms the ethno-pharmacological importance of *G. kola* and *C. nitida* and could serve as the basis for advanced studies in the development of drugs against infectious diseases. This would also prove useful especially due to the alarming rate of drug resistance which is posing a threat and a major challenge in treatment of infectious diseases. Apart from performing synergistic studies to evaluate the performance of *G. kola* and *C. nitida* when combined with orthodox medicine, there is also a need to determine the toxicity of the plant extracts which in our findings will be a prelude to initiating clinical trials in subsequent drug development.

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