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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *GARCINIA KOLA* (HACKEL) AND *COLA NITIDA* (VENT) EXTRACTS

B. T. Afolabi[1,*], G. C. Agu[2] and I. B. Onajobi[3]
[1, 2, 3,] DEPT. OF MICROBIOLOGY, OLABISI ONABANJO UNIV., P. M. B. 2002, AGO-IWOYE, OGUN STATE, NIGERIA.
E-mail addresses: [1] *oluwabukolaafolabi14@yahoo.com,* [2] *georgiaagu@yahoo.com,*
[3] *onaobii@yahoo.com*

ABSTRACT
This study was designed to evaluate the phytochemical and antibacterial properties of Garcina kola (Orogbo) and Cola nitida (Obi) extracts. Fresh seeds of Cola nitida and Garcinia kola were collected from Ago-Iwoye market, Ogun State. The ethanolic and aqueous extracts of the test plants were used against selected test organisms, Streptococcus pneumonia, Staphylococcus aureus, Salmonella typhi, Pseudomonas euriginosa and Escherichia coli. Disc diffusion method was adopted to test for susceptibility of the selected test bacteria to the extracts. Minimum inhibition concentration (MIC) and Minimum bactericidal concentration (MBC) were determined. Bioassay data were statistically analyzed using two-way ANOVA. The result of the phytochemical screening confirmed the presence of flavonoid, phenol, alkaloid, saponins, tannins and phlobatanins. The result of the antibacterial activity showed that the ethanolic extract of the test plants recorded highest antimicrobial activity against test isolates compared to aqueous extracts. The tested plant seeds of both plants posses reasonable antibacterial activity but to varied zones of inhibition, with Staphylococcus aureus isolate having the highest inhibitory zones (21.33) mm while Pseudomonas aeuruginosa had the least inhibitory zone (6.00) mm. The antibacterial activity were however found to be concentration dependent (Fvalue= 3.996, Pvalue= $<$ 0.05). All tested organisms were found to have definite MIC and MBC activities which ranges between 125 and 1000µg/ml for MIC except for Kola nitida that has no definite MBC below 1000µg/ml. The result confirms the potential of antibacterial activity of Garcinia kola and Cola nitida extracts.

Keywords: *Antimicrobial, Cola nitida, Garcinia kola, Phytochemical, Plant extracts*

1. INTRODUCTION

The use of herbs has a long history in health care delivery in Africa. According to World Health Organization [26], the evolving public health threat of antimicrobial resistance is driven by both appropriate uses of anti-infective medicines. The scientific evaluation of traditional drugs of plant seed origin and screening of more effective and safe hypoglycemic agents has continued to gain medicinal importance stated by Biswas [3]. The medicinal values of many of these seed cannot be over emphasized in the light of oral traditions and folklores from the distance past that have continued to extol the healing virtues of these seeds and their extracts according to Amabeoku and Kinyua [2].

Medicinal plants can be regard as the richest bio-resource of drug of modern medicine, folks medicine and chemical entities or templates for synthetic drugs by Joshua and Takudzwa [13]. The discovery of medically important metabolites in common and abundant plant would minimize over exploitation of well known, rare medicinal plants.

Plant seeds contain bioactive components such as flavonoids, glycosides, saponins and tannins according to Tiwari *et al* [23], which possess medicinal properties that are harnessed for the treatment of different diseases stated by Prohp *et al* [19]. Dietary plant seeds with proven antioxidant properties may function as a direct anti-radical chain breaker of free radical propagation, interaction with

* Corresponding author, tel: +234 703 364 2713

transition metals and inhibition of Reactive Oxygen Species (ROS) generating enzymes postulated by Hassan *et al* [8]. The use of plants for medicinal purposes continues to this day, usually in the form of traditional medicine, which is now recognized by the World Health Organization as a building block for primary health care. Well-known examples of drugs with plant origins includes aspirin, atropine, digoxin, ephedrine, morphine, quinine, reserpine, vincristine and vinblastine, as well as several plant steroidal sapogenins which serve as semi-synthetic precursors to the steroidal drugs according to Lim [16].

The plant *Garcinia kola* is one of such plants. *Garcinia kola*, commonly called bitter kola is found mainly in tropical rain forest region of Central and Western Africa by Check [5]. *Garcinia kola* which is also known as bitter cola is highly used for its medicinal purposes because of its anti-viral, anti-inflammatory, anti-diabetic, bronchio-dilator and anti-hepotoxic attributes. Obi and Adaipoh[18] postulated that Fruit extracts from *G. kola* have proven effective at stopping Ebola virus replication in laboratory test. The sap from *Garcinia kola* is used for the treatment of parasitic skin diseases while the latex is orally ingested for the treatment of gonorrhea. It is also useful in the eradication of guinea worm infestation.

Meanwhile, *Cola nitida* is among various species of cola, they are eaten by elderly people. The greatest concentration of *Cola nitida* is in the forest area of Ivory Coast and Ghana. *Cola nitida* is the kola which has social and traditional significance. Kola contains about two percent caffeine and is chewed by many people as a stimulant. It is used in the manufacture of dyes, it is also used in the manufacture of the cola group beverages- coca-cola, Pepsi cola and kola. Plants have been used since antiquity for medicinal purposes by diverse peoples and cultures throughout the world according to Lim [16]. This present study evaluated the phytochemical and antibacterial properties of *Garcina kola* (Orogbo) and *Cola nitida* (Obi) extracts.

2 METHODOLOGY

Aseptic techniques and universal laboratory safety protocols were followed throughout the course of the experiment.

2.1 Plant Collection

Fresh seeds of *Cola nitida* and *Garcinia kola* were collected from Ago-Iwoye market, Ijebu North Local Government of Ogun State. These seeds were authenticated at Forestry Research Institute of Nigeria, Jericho, Ibadan, Oyo State (FRIN).

2.2 Processing of samples

The samples were washed under running tap and allowed to dry at room temperature. The dry sample were cut into smaller particles and air-dried at room temperature for two weeks and ground using an electric blender (Lexus MG2053, India) to obtain small size materials (approximately 2-4 mm) in size as also reported by Karthy *et al*[14].

2.3 Plant Extraction

Extracts were prepared by maceration of the powered samples (250g/ plant) in absolute 500 ml ethanol for 72 hours and 500 ml deionized water for 24 hours with intermitted shaking. The extracts were filtered using Whatman grade 1filter paper while filtrates from the ethanol extracts were obtained and concentrated *in-vacuo* using rotary evapora
or (BUCHI Rotavapor R-250 EX, India) at 45^{o}C and the waxy concentrate were stored at temperature 4^{o}C until use. Furthermore, filtrates from aqueous extracts were dried using vacuum freeze-dryer (Esquire Biotech, FD-18-MTP, India) and the powdered sample were collected and stored at room temperature. Concentrated extracts were weighed to find the extraction efficiency on dry weight basis. Extraction efficiency was calculated as follows;

Extraction efficiency% = (Final dry weight of extract/ Initial weight of dried plant material) $\times$ 100 described by Shilpakar *et al* [21].

2.4 Test organisms

Five organisms including *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli* and *Streptococcus pneumonia* were used as test organisms. The organisms were obtained from University Teaching Hospital (UCH) Ibadan, Oyo State. These organisms were further streaked on nutrient agar and incubated at 37^{o}C for 18 hours respectively. The isolates identities were further confirmed using standard biochemical procedures as described by Leber[15], the isolates were stored on agar slant at 4^{o}C prior to their use. The cultured organisms were suspended into nutrient broth and this was standardized according

to NCCL[17]. By adding normal saline gradually in order to compare its turbidity to McFarland standard of 0.5 this is approximately 1.0× 106 Cfu/ml.

2.5 Phytochemical analysis

Each extract was investigated for the presence of secondary metabolites like alkaloids, anthraquinine glycosides, cardiac glycosides, flavonoids, saponins, cardenolide, phenol, tannins, carbohydrates using qualitative standard method of Trease and Evans [24].

For Alkaliods, one gram of the dried powdered sample was heated with 10 mL of 10% HCl on a water bath for five minutes. The extract was then filtered and allowed to cool. The pH was adjusted to about 6-7 by adding 10% ammonia and using litmus paper. The presence of turbidity or precipitation indicated presence of alkaloid. For anthraquinone glycosides, the borntrager's test: One gram of powdered sample were heated with 2 mL of 10% HCl by boiling for five minutes and filtered while still hot, then allowed to cool. The filtrate was partitioned with equal volume (aliquot) of chloroform and mixed gently. The presence of delicate rose-pink layer on the test solution indicated the presence of anthraquinones glycosides.

For flavonoids, one gram of powdered sample were added to 10mL of ethanol and 3 drops of $FeCl_3$ solution was added, dark green colour observed indicated the presence of flavonoid. One gram of powdered sample were added to distilled water and heated for 3 mins. It was allowed to cool and 2 mL conc. H_2SO_4 was added, yellow coloration disappear which shows the presence of flavonoid.

For tannin, the Braemer's test**:** One gram of the powdered sample were decocted with 10 mL of distilled water by boiling for 10 minutes and filtered while hot and allowed to cool. 0.1% Ferric chloride reagent was added to the filtrate and observed. A blue-black, green or blue green precipitate indicated presence of tannins.

For saponins, Frothing**:** One gram of powdered sample were transferred into a test tube containing 10 mL of distilled water and then boiled for five minutes and then filtered. The filtrate was mixed vigorously and observed. The presence of froths indicated the presence of Saponins. The mixture was shaken vigorously and kept for 3min after which was observed. A honey comb like froth was formed and it showed the presence of saponins.

For phenols, one gram of powdered of sample were added to 10 mL of ethanol and 3 drops of phenol solution were added. A dark green colour observed indicated the presence of phenol. For steroids, two ml of acetic anhydride were added to 0.5 g of ethanol extract of each sample with 2 mL H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

For Terpenoids (Salkowski test)**,** five ml of ethanol extract were mixed in 2 mL of chloroform and 3 mL concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the inter-phase formed indicated a positive result for the presence of terpenoids.

For Phloba-tanins, one gram of powdered sample were transferred into a test tube containing 10 mL of distilled water and then boiled for five minutes and filtered. To the filtrate 5 mL of 1% HCl and boiled for 5 mins. The presence of precipitate is a positive result.

2.6 Antibacterial Assay

Antibacterial activity of the extracts was carried out by using overnight cultures of the tested bacteria. An already solidified sensitivity test medium on petri dishes were seeded with the test organisms using sterile swab stick (spread plate method) after which four wells were made using cork-borer (6mm). The extracts were reconstituted by adding 0.2 mL of ethanol to 0.0001g of the paste like form of *G.kola* and *C.nitida* after proper mixing, 1mL of distilled water was then added. Various concentrations of 125, 250, 500 and 1000 µg/mL were obtained. The plates were incubated at 37^0C for 24 hours and the zones of inhibition were measured in millimeter using transparent meter rule. Antibiotics sensitivity disc were used as positive control, the test organisms were also prepared using spread plate method, while ethanol and water serves as negative control. Each antibacterial assay was carried out in triplicate.

2.7 Determination of minimum inhibitory concentration (MIC)

A serial dilution ranges of the extracts to obtain concentrations (0.99, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250,500 and 1000 µg/mL) were made to determine the MIC. The bacterial strains were cultured in Nutrient broth. In the micro plate (96-well U-Bottom (50063), Gaithersburg) 1.0 mL of test concentrations of each was added to 1.0 mL

suspension of microorganism and incubated at 37°C for 24 hrs.

2.8 Determination of minimum bactericidal concentration (MBC)

Suspensions from the MIC studies were used for the MBC determination to a solid media a bacterial streaking of equal streaks of the suspension from the MIC was made and the procedures were repeated all through the required numbers of the corresponding isolates. The concentrations were incubated at 37°C for 24 hrs. After incubation, the plates were observed.

3 RESULTS

The result of the phytochemical analysis of the seeds (*Garcinia kola* and *Cola nitida*) is presented in Table 1 below. This analysis shows the presence of alkaloids, saponin, tannin, anthraquinone glycoside, cardiac glycoside, phenol, steroids and so on. The antimicrobial properties of the seeds have been correlated to the presence of secondary metabolite.

3.1 Results on Antibacterial Assay

Table 2 below depicts the antibacterial activity of ethanolic extract of *Garcinia Kola* exhibits varying degrees of activities against the tested bacterial isolates, with *Staphylococcus aureus* having the highest sensitivity patterns (15.333 + 0.00 to 21.333 + 0.00). *Pseudomonas aeruginosa* was however found to be the least sensitive isolates of the tested isolates. Except the strain of *Pseudomonas aeruginosa* tested in this study, all other tested bacterial isolates exhibited concentration dependent activities in increasing order of 125, 250, 500 and 1000 µg/mL respectively.

The inhibitory effects of ethanolic extract of *Cola nitida* on growth of test organisms exhibit no degree of activity, likewise aqueous extract of both *Garcinia kola* and *Cola nitida* shows ineffectiveness on the tested bacterial species.

Table 4 shows the inhibitory effects of some antibiotics against tested organisms using sensitivity disc. *E. coli*, *S. pneumonia*, *S. typhi* and *P. aeruginosa* are most sensitive to Ciprofloxacin while *S. aureus* was sensitive to Septrin.

The minimum inhibitory concentration (MIC) of both *Garcinia kola* (GK) and *Cola nitida* (CN) results obtained in this study shows that both plant extracts have a definite minimum inhibitory concentration. *Salmonella typhi* was however found to be the most sensitive to GK as demonstrated by its lowest MIC value. On the other hand, *Streptococcus pneumoniae* had the lowest MIC to CN, depicting it to be the most sensitive. The lowest activity to the tested plants observed were *Staphylococcus aureus* and *E.coli* to GK and CN respectively. *Salmonella typhi* also had very high MIC in CN as presented below

Table 6 connotes the Minimum Bactericidal Concentration (MBC) of both *Garcinia kola* and *Cola nitida*. Result obtained in these table shows that GK have minimum bactericidal concentration but *streptococcus pneumonia* has the highest MBC to GK. On the other hand, *E.coli* had the lowest MBC to CN, depicting it to be the most sensitive of the tested isolates to CN while *streptococcus pneumonia, Salmonella* tyhpi*, Pseudomonas aeruginosa* and *Staphylococcus aureus* are greater than 1000 µg/ml.

4 DISCUSSION

This study evaluates the phytochemical, antibacterial efficacy of *Garcinia kola* and *Cola nitida* on clinical isolates. Results of phytochemical analysis of *Garciniakola* seeds and *Cola nitida* revealed the presence of flavonoids, tannins, glycosides, saponins, alkaloid, anthraquinone glycoside, at different concentrations, which correlates with work of Udenze *et al* [25] who also reported the presence of active ingredients in their research work on these extracts. *G. kola* seed is rich in bioflavonoids according to Terashima *et al* [22] and has been speculated to stimulate the immune system because of its antioxidant and other related activities demonstrated by the seed extracts in respect to[Iwu *et al*, Farombi *et al* and Adaramoye *et al*][12, 6 and 1]. For *Cola nitida*, flavonoids, starch, terpenoids and phlobatins are absent and it is related to the work of Biswas[3] who also repeated the absence of some phytochemical constituent. These partly explain the general acceptance and high success rates claimed in ethnomedicinal uses of *G. kola* and *Cola nitida* seeds in inflammatory and infective conditions [Hussan *et al*, Iwu *et al*, Iwu *et al*, Braide *et al* and Iwu *et al]* [9, 10, 11, 4 and 12].

Table 1 Result of phytochemical screening

Test	GK	CN
Alkaloid		
- Mayers reagent		++
- Wagner reagent	++	++
- Drangendoff reagent	++	++
	++	-
Anthraquinone glycoside	-	
Cardiac glycoside	-	-
Tannins	++	+
Saponin		
- Frothing	++	+
- Emulsification	++	+
- Na_2CO_3	++	+
Flavonoid		
- Ethanol	++	-
- EtOAc	++	-
- H_20	-	-
Phenol	++	+
Steroids	-	-
Starch	-	-
Terpenoids	-	-
Phlobatanins	+	-

Keys: + = trace ++ = moderate - = absent
GK= *Garcinia kola* CN= *Cola nitida*.

Table 2 Inhibitory effects of Garcinia kola ethanolic extract against test organisms Size of coke- borer = 6mm

Test Organisms		Concentration		
125 µg/mL		250 µg/mL	500 µg/mL	1000 µg/mL
S. pneumonia	7.0000+1.00	9.0000 +1.53	11.0000 +2.52	14.6667+1.76
S. aureus	15.333 + 0.00	16.667 + 0.00	17.667 + 0.00	21.333 + 0.00
S. typhi	11.3333+ 0.88	13.6667+0.88	16.0000+1.15	25.3333+2.73
P.aeuruginosa	6.0000+ 0.00	6.0000+ 0.00	6.0000+ 0.00	6.0000+ 0.00
E. coli	7.3333 + 0.88	10.0000+1.15	11.6667+1.33	12.3333+1.20

Table 3 Inhibitory effects of Cola nitida on ethanolic extract against test organism. Size of coke- borer = 6mm

Organisms	125 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL
S. Pneumonia	6.0000±0.00	6.0000±0.00	6.0000± 0.00	6.0000± 0.00
S. aureus	6.0000± 0.00	6.0000± 0.00	6.0000± 0.00	6.0000± 0.00
S. tyhpi	6.0000± 0.00	6.0000± 0.00	6.0000± 0.00	6.0000± 0.00
P. auginosa	6.0000± 0.00	6.0000± 0.00	6.0000± 0.00	6.0000± 0.00
E. coli	6.0000± 0.00	6.0000± 0.00	6.0000± 0.00	6.0000± 0.00

This research work showed that *G. Kola* and *C. nitida* possesses some degree of inhibitory effects against the tested isolate. It has also been identified to have strong antibiotic activities and found to be very effective against disease causing microorganisms.

The Minimum Inhibitory Concentration of *Garcinia kola* (GK) and *Cola nitida* (CN). Results obtained in this study showed that both plant extracts have a definite MBC. Inhibitory effects of some antibiotics against tested organisms using sensitivity disc.

Table 4 Zones of inhibition of Antibiotics against Test organisms (positive control on the growth of bacteria) Size of disc- 8mm

Antibiotics(µg)	E.coli	*S.pneumoniae*	*S.aureus*	*S. typhi*	*P. aeruginosa*(mm)
Septrin (30)	11.00	15.00	14.00	10.00	-
Chloramphenicol(30)	-	-	-	18.00	-
Sparfloxacin(10)	20.00	-	-	18.00	12.00
Ciprofloxacin(10)	22.00	23.00	10.00	23.00	20.00
Amoxicillin(30)	-	10.00	10.00	-	-
Augmentin (25)	-	-	-	-	-
Gentamycin(10)	-	16.00	-	10.00	-
Pefloxacin(10)	13.00	17.00	10.00	19.00	10.00
Tarivid(30)	16.00	-	13.00	20.00	18.00
Streptomycin(30)	-	18.00	-	10.00	-
Ampiclox(30)	-	10.00	11.00	-	-
Zinnacef(20)	-	20.00	-	-	-
Rocephin(25)	-	17.00	12.00	-	-
Erythromycin(10)	-	20.00	-	-	-

Table 5 Minimum Inhibitory Concentration (MIC)

Organisms	GK (µg/mL)	CN (µg/mL)
S. pneumonia	10.42	10.42
S. typhi	7.81	41.67
P. aeruginosa	31.25	31.25
E. coli	31.23	41.67
S. aureus	12.5	12.5

Keys: GK- *Garcinia kola*, CN-*Cola nitida*

Table 6 Minimum Bactericidal Concentration (MBC)

Organisms	GK (µg/mL)	CN(µg/mL)
S. Pneumoniae	250	> 1000
S. typhi	125	>1000
P. aeruginosa	125	>1000
E.coli	125	250
S. aureus	125	>1000

Keys: GK- *Garcinia kola*, CN: *Cola nitida*

E. coli, *S. pneumonia*, *S. typhi* and *P. aeruginosa* are most sensitive to Ciprofloxacin while *S. aureus* was sensitive to Septrin. *Salmonella typhi* was however found to be the most sensitive to GK as demonstrated by its lowest MIC value. On the other hand, *Streptococcus pneumoniae* had the lowest MIC to CN, depicting it to be the most sensitive.

Report shows that ethanol is preferred by many researchers since it poses less toxicity and higher extraction yield according to Franco *et al* [7]. Rahmoun *et al*[20] States that extraction yield increases with the increasing polarity of the solvent which is in agreement with the derived output.

5 CONCLUSION

The results of this study revealed that the tested plant extracts possesses reasonable antibacterial activity against the test bacterial isolates. The extracts were more effective on *Staphylococcus aureus* than other test bacterial isolates. Though, the antibacterial activities were found to be concentrated dependent. The plants extracts could serves as treatment choice for *Staphylococcus aureus* infection in the nearest future. Further work need to be carried out to ascertain the proactive components present in *Cola nitida* and *Garcinia kola* extracts for future use.

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