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Assessment of the Phytochemical and Antibacterial Profiles of Aqueous and Ethanolic Extracts of *Garcinia Kola* Seed

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Abstract

Plants remain a major resource in traditional medicine, with growing evidence of their therapeutic effectiveness. Phytochemical screening of medicinal plants is therefore vital to identify potential sources of bioactive compounds for pharmaceutical applications. This study investigated the phytochemical constituents and antibacterial activity of aqueous and ethanolic extracts of *Garcinia kola* seed. Standard techniques were employed to detect tannins, phlobotannins, saponins, steroids, terpenoids, phenolics, flavonoids, alkaloids, resins, and glycosides. The extracts revealed the presence of alkaloids, tannins, saponins, flavonoids, phenolics, glycosides, phlobotannins, and resins. Antibacterial activity was evaluated using the agar-well diffusion method against *Proteus* sp., *Escherichia coli*, *Enterobacter* sp., and *Erwinia* sp. Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined by two-fold serial dilution. Both ethanolic and aqueous extracts at 400 mg/mL inhibited the test organisms. *E. coli* showed the least inhibition zones (5.85 ± 0.15 mm and 9.33 ± 1.25 mm for aqueous and ethanolic extracts, respectively), while *Erwinia* sp. exhibited the highest inhibition (8.00 ± 1.0 mm and 15.75 ± 2.20 mm, respectively). The ethanolic extract consistently demonstrated stronger activity than the aqueous extract. MIC values ranged between 200 and 400 mg/mL for the ethanolic extract, while MBC was observed at 400 mg/mL. MIC values were lower than MBC, indicating that the extracts are primarily bacteriostatic at lower concentrations and bactericidal at higher ones. Inhibitory effects differed significantly ($P < 0.05$) among extracts and controls. The findings suggest that *G. kola* seed possesses valuable phytochemicals with antibacterial properties, supporting its use in traditional medicine. Further research and funding are recommended to isolate and characterize the active compounds, which may provide effective alternatives in the treatment of modern-day infections.

Keywords: *Antibacterial, Antimicrobial resistance, Garcinia kola, Pharmaceutical industries, Phytochemical*

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1. Introduction

The multiple antimicrobial resistance bacteria cause severe problems that results in complication of treatment of bacterial infections and this has been recognized by the World Health Organization, (WHO, 2001) (Omwirhiren *et al*., 2017). Antibiotics are used and were believed to lead in the complete eradication of infectious diseases. Despite the progress made in introducing new antibiotics, the emergence of drug resistant strains causes failure of infectious disease treatment. Studies have shown that antibiotic resistance occur as a result of an intrinsic mechanism that prevent bacteria from destruction (Omwirhiren *et al*., 2017). These bacteria usually do not have the structural cellular mechanism that are needed in order for the antibiotic to act upon (Mannetti *et al*., 2007).

In order to face all these health problems, the formalization of endogenous knowledge would be a reliable asset in the control of these resistant microbial strains. Also, the management of resistant bacteria is an attractive strategy using medicinal plants. Medicinal plants cater for about 80 % of the vast populace that rely mostly on herbs for their medicines (Temitope *et al*., 2016; Neethu *et al*., 2016). The medicinal values of plants lie in the chemical substances presents in the parts of the plant such as seed, leaves bark and root. These substances produce definite

physiological action in the human body (Ukaoma *et al*., 2013). It is known that several medicinal plants synthesize a wide variety of phytochemicals which include alkaloids, tannins, flavonoids, steroids, saponins, and phenols, which have antimicrobial proprieties. As a result, many plants were used as a source of traditional medicine to treat various diseases and conditions (Dah-Nouvlessounon *et al.*, 2015). Medicinal plants may offer a new source of antibacterial, antifungal and antiviral activities. Studies have shown that they have less side effects, less expensive and effective against broad spectrum drug resistant microorganisms (Maiyo *et al*., 2010).

Garcinia kola, often called bitter kola, is an indigenous medicinal tree belonging to the family *Guttiferae*. It is commonly called Namiji goro in Hausa, Akilu in Igbo and Obi in Yoruba. Morphologically, *Garcinia kola* resembles *Allanblackia floribunda*. It is well branched, evergreen, and grown as a medium size tree, reaching 12 m high in 12 years, and found in moist forests throughout West and Central Africa. *Garcinia kola* has regular fruiting cycle and the tree produces fruits every year. Garcinia kola is popular in South - Eastern Nigeria as it is extensively used in herbal medicine. It is one of the most important trees valued in Nigeria for its medicinal seeds and its exploitation in the natural forests has been very heavy (Anagbeh *et al*., 2006). It is used in traditional medicine for various therapeutic purposes based on pharmacological effects of the active components (Biflavonoids, Xantones, Benzophenones, Kolaviron and flavonones) in the seed and other parts of the plant such as the stem bark (Farombi *et al*., 2005).

Previous studies by Ukaoma *et al*. (2013) reported that bark extract of *Garcinia kola* was more effective in inhibiting the growth of *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* when compared with the extract from the root and seed. In Nigeria, there are few scientific studies on this plant species *Garcinia kola* despite the widespread use of medicinal plants. It is in the light of this background that the study was designed specifically to evaluate the phytochemical and antibacterial profiles of aqueous and ethanolic extracts of *Garcinia kola* seed.

2. Materials and Methods

2.1 Study Site

The fresh seeds of *Garcinia kola* were collected from Eke-Awka Market, Awka, in Awka South Local Government Area, Anambra State.

2.2 Preparation of Seeds for Extraction

The seeds were cut into smaller portions and dried under shade at room temperature for 14 days. The dried seeds were grounded using electric grinder model to powdery form, weighed and kept ready for extraction of active ingredients as in (Umeh *et al*; 2021).

2.3 Extraction Procedure

A 20g portion each of the seed powder was extracted by maceration in 200 mL each of ethanol and water for 24hours. The resulting extracts were subsequently filtered using Whatman No. 1 filter paper. The ethanolic extracts was evaporated to dryness at room temperature in a steady air current (Okoye *et al*; 2014). The aqueous extract was evaporated in a water bath at 100°C to dryness. The standard extracts obtained were stored in a refrigerator at 4°C until required for use.

2.4 Phytochemical Studies

The phytochemical constituents of the seed's extracts were investigated using the methods used by Sofowora, (1993) and Ezeonu, and Ejikeme, (2016). The phytochemical tests are of paramount importance in order to identify the various constituents in the extracts respectively responsible for the various pharmacological actions elicited by the extracts as follows: tannins; phlobatannins, saponin, steroid, terpenoids, flavonoids, alkaloids and glycoside, respectively.

2.5 Preparation of Test Sample

In this study, concentration of 400 mg/mL of each extract was used for the screening of antibacterial activity. This was done by dissolving 4.0 g of the extract in 10 mL of solvent (NCCLS, 2000).

2.6 Collection of Clinical Isolates

Pure isolates of *Proteus* sp., *E. coli, Enterobacter* sp., and *Erwinia* sp. was collected and authenticated in Microbiology Laboratory of Pharmaceutical Microbiology and Biotechnology Department, Nnamdi Azikiwe University, Agulu and the identity was reconfirm using appropriate colonial characteristics, staining techniques and biochemical test according to the method of Cheesbrough (2006). For the experiments, the bacterial isolates were first subcultured in nutrient broth and incubated according to standard procedure. Pure colonies of the bacterial isolates were characterized and identified using Bergey's manual of Determinative Bacteriology authored by (Holt et at., 1994) after carrying out the biochemical test (Gram staining, spore staining, motility test, catalase test, citrate test, indole test, Methyl red test, Voges Proskauer test and sugar fermentation test).

2.7 Preparation of 0.5 McFarland standards

Solution A was prepared by adding 1.175 g of barium chloride ($BaCl_2$, $2H_2O$) to 100 mL 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 (Omwirhiren *et al*., 2017).

2.8 Antibacterial Bioassay

This was carried out using agar well diffusion techniques. Each of the labeled medium plates was uniformly inoculated with the test organisms using pour plating technique. A sterile cork borer of 5mm diameter was used to make wells on the medium. Then, 0.1 mL of the various extracts concentrations was dropped into each labeled well (Mundi *et al*; 2014). After that, the plates were incubated at 37 °C for 24 hours. The antibacterial activity was determined by measuring the diameter zones of inhibition (mm) produced after incubation. 0.05 % ciprofloxacin was used as a control (NCCLS, 2000).

2.9 Determination of Minimum Inhibitory Concentration (MIC)

This was achieved by assaying different concentrations of the stock; 400, 200, 100, 50, 25, 12.5 mg/ml against the test organisms. The MIC was the lowest concentration that was able to inhibit any visible bacterial growth (Okoye *et al*; 2014).

2.10 Determination of Minimal Bactericidal Concentration (MBC)

Here, equal volume of various concentrations of those tubes that did not produce any growth for MIC were sub cultured on fresh sterile poured plate and incubated at 37°C for 24hours. The lowest concentration that yields no single bacterial colony was the MBC (Okoye *et al*; 2014).

2.10 Statistical Analysis

The results were expressed as mean ± standard deviation (mean ± s. d.) of three different measurements. Statistical analysis was performed on data generated from the study using Microsoft excel and SPSS softwares. Statistical significance was determined using one way analysis of variance (ANOVA) and student's t-test. Values with $P < 0.05$ compared with the control groups were considered as being significantly different (Uba, 2018; Uba *et al*. 2018; Njoku *et al*. 2019; Uba, 2019; Uba *et al.*, 2019a; Ibo, *et al*., 2020).

3. Results and Discussion

The result of the preliminary phytochemical analysis of *Garcinia kola* seed extract is shown in Table 1. The ethanolic seed extract of *Garcinia kola* possesses alkaloids, tannins, saponins, flavonoids phenolics, glycosides, phlobatannins and resins while the aqueous extract possesses alkaloids, tannins, saponins, flavonoids and resins. The ethanolic seed extract of *Garcinia kola* contains more of tannins, saponins, phenolics and less of alkaloids, phlobatannins and resins. The aqueous seed extract does not contain phenolics, glycosides, phlobatannins and steroids is similar to the work of Jackie *et al.* (2014), Anameze *et al*. (2023) and Ibrahim *et al*. (2024) who reported the presences of tannins, saponins, flavonoids, terpenoids, glycosides and alkaloids with the absence of steroids and phenols.

Table 2 presents the morphological and biochemical characteristics of four bacterial isolates: *Proteus* sp., *E. coli*, *Enterobacter* sp., and *Erwinia* sp. Each isolate has distinct colony morphology, such as swarming growth for *Proteus* sp. and smooth, opaque colonies for *E. coli*. All isolates are Gram-negative rods. *Proteus* sp. and *Enterobacter* sp. can utilize citrate, indicated by a positive reaction. All isolates are motile with only *E. coli* is indole-positive. *Proteus* sp. is urease-positive, while the others are negative. The isolates show varying patterns of sugar fermentation, which can be used for identification. These strains have been implicated in antibacterial studies by several researchers (Okoye *et al*. 2016a; 2016b; 2020a; 2020b; 2020c; Uba *et al*. 2017; 2018; 2019b; Dokubo *et al*. 2022). These characteristics were used to confirm the identities of the clinical bacterial strains collected before proceeding to the antibacterial activity proper.

Antibacterial studies are conducted to determine efficacy and potency of chemotherapeutic agents. Interest in higher plant extracts exhibiting antimicrobial activity has increased in recent years, and several reports on this subject have been published (Iheukwumere *et al*. 2012a; 2012b; Mundi *et al*. 2013; 2014; Okoye *et al*. 2014; Uba *et al*. 2016; Umeh *et al*. 2021; Ele *et al*. 2025; Okeke *et al*., 2025). In this study, the preliminary antibacterial activity of *G. kola* seed extracts was reported in the Table 3. The antibacterial activity of ethanolic seed extract of *G. kola* is presented in Table 4 while Table 5 showed the antibacterial activity of aqueous seed extract of *G. kola*. The higher concentrations of the extracts inhibited activities against the four tested organisms: *Proteus* sp., *E. coli*, *Enterobacter* sp. and *Erwinia* sp. which are Gram – negative (Table 3). The investigations done on *G. kola* extracts revealed that the plant possesses antimicrobial activities against the tested bacterial isolates at a final concentration of 400 mg/mL. Also, *E. coli* showed the least zone of inhibition of 5.85 $\pm$ 0.15 and 9.33$\pm$ 1.25 mm while *Erwinia* sp. had the highest zone of inhibition of 8.00$\pm$ 1.0 and 15.75$\pm$ 2.20 mm of both aqueous and ethanolic extracts respectively (Tables 4 and 5). The ethanolic extract demonstrated stronger activity than the aqueous extract and was significantly different ($P < 0.05$) at concentrations of 100, 200, and 400 mg/mL of the extract against the tested organisms. At concentration of 50 mg/mL, it reacted significantly against all the tested organisms except *E. coli*, but the 25 mg/ml was not significant on all the tested organisms (Table 4) but not so in aqueous extract (Table 5) where only the concentration of 400mg/ml could show significant activity against all the tested organisms, 200 mg/mL was active against *Proteus* sp., *Enterobacter* sp. and *Erwinia* sp., 100 mg/mL was active only against *Erwinia* sp. while the concentrations of 50 mg/mL and 25 mg/mL did not show significant activity against all the tested organisms. This implied that the active antibacterial compounds resided more in ethanolic fraction and that antimicrobial activity of the extracts increased with concentration. This observation is similar to the works carried out by several researchers (Adegboye *et al*., 2008; Ukaoma *et al*. 2013; Dah-Nouvlessounon *et al*. 2015; Omwirhiren *et al*., 2017). On the other hand, zones of inhibitions exhibited by ciprofloxacin as standard/control antibiotic used ranged between 22.00$\pm$ 1.48 mm and 27.50$\pm$ 1.56 mm and found to be greater than all the *G. kola* extracts. From this observation, *G. kola* extract could not be compared with the standard/control antibiotic ciprofloxacin.

The MIC of the seed extracts against the tested bacterial isolates was also determined. The results of the minimum inhibitory concentration of *G. kola* seed extracts is reported in Table 6. The MIC varied between 200 and 400 mg/mL of ethanolic extract against the test organisms. It was observed that the four test organisms: *Proteus* sp., *E. coli*, *Enterobacter* sp. and *Erwinia* sp. tested against the aqueous extract did not show any growth after 24 hrs of incubation. Unfortunately, the reason for this was not determined. The standard or control antibiotic, ciprofloxacin had MIC values varying between 25 and 100 mg/mL. The results indicated that the standard or control antibiotic ciprofloxacin possesses stronger activities than both extracts of *G. kola* as shown in Table 6. From all indications, if the ethanolic crude extract becomes more purified, the activities might look stronger than that of the crude extract and might have compete favourably in activity with the standard antibiotic. This observation is similar to the work done by Adegboye *et al*., (2008) where they found that standard antibiotic streptomycin had greater activity than the crude extract of *G. kola.*

The MBC of the *G. kola* seed extract against bacterial isolates was also determined (Table 7). MBC value was 400 mg/mL for ethanolic extract only with similar observation like MIC. Comparatively, the MIC was lower than the MBC indicating that the extracts are mostly bacteriostatic at lower concentrations and bacterial at higher concentrations. This observation is in agreement with research done by Nwaokorie *et al*., (2010) that found that the MIC was lower than the MBC except for the amoxicillin resistance strain when *G. kola* was tested against biofilm produced by the association of *F. nucleatum* isolates. The standard/control antibiotic ciprofloxacin had MBC values varying between 50 and 200 mg/mL, respectively. The results indicated that the standard control antibiotic possesses stronger activities than both extracts of *G. kola*.

The antibacterial activity of the G. *kola* seed extracts can be attributed to the synergistic action of some bio reactive substances such as the alkaloids, tannins, saponins, flavonoids among others in the extracts. Many higher plants are known to possess antibacterial agents and indeed extracts of plants from different parts of the world have been known to produce antimicrobial properties as observed in this work (Ukaoma *et al*., 2013).

Table 1: Preliminary phytochemical analysis of *Garcinia kola* seed extracts

Phytochemical	Ethanolic extract	Aqueous extract
Alkaloids	++	+
Tannins	+++	+
Saponins	+++	++
Flavonoids	++	++
Phenolics	+++	-
Glycosides	++	-
Phlobotannins	+	-
Renins	+	++
Steroids	-	-

Key: + = Low; ++= Moderate; +++= High; - = Negative

Table 2: Morphological and Biochemical Characteristics of the isolates

Test	*Proteus* sp.	*E.coli*	*Enterobacter* sp.	*Erwinia* sp.
Cultural	Swarming growth, Fishy smell	large, circular opaque, smooth moist	large, thick grayish white smooth	white,smooth, colonies, domed, shining, mucoid, entire
Gram reaction	-	-	-	-
Morphology	rod	rod	rod	rod
Simon Citrate	+	-	+	+
Motility	+	+	+	+
Indole	-	+	-	-
Urease	+	-	-	-
Spore test	-	-	-	-
Catalase	+	+	+	+
Methyl red	+	+	-	+
Voges proskauer	-	-	+	+
Glucose	+	+	+	-
Lactose	-	+	-	-
Sorbitol	-	+	+	-
Maltose	-	-	+	-
Fructose	-	-	+	+
Sucrose	-	-	+	+
Raffinose	-	-	+	+

+ =Positive; - = Negative.

Table 3: Preliminary antibacterial activity of *Garcinia kola* seed extracts

	Diameter zones of Inhibition (mm) of the various microorganisms			
Extract	*Proteus* sp.	*E. coli*	*Enterobacter* sp.	*Erwinia* sp.
EEG	12.75 ± 0.05	9.33±0.03	15.25 ±0.02	15.75 ± 0.05
AEG	7.37 ± 0.03	5.85± 0.02	7.50 ± 0.01	8.00 ± 0.01
CPX (0.50%)	27.50 ± 0.04	22.00± 0.01	26.70 ± 0.01	25.80 ± 0.01

Values are mean ± S.E.M of triplicate measurements. Concentration of extracts are in 400 mg/ml; CPX = Ciprofloxacin; EEG = Ethanolic extract of *Garcinia kola;* AEG = Aqueous extract of *Garcinia kola*. Unit of extract is in mg/ml.

Table 4: Antibacterial activity of ethanolic seed extract of *Garcinia kola*

	Diameter zones of inhibition (mm)			
Extract	*Proteus* sp.	*E. coli*	*Enterobacter* sp.	*Erwinia* sp.
400	12.75 ± 0.05	9.33± 0.03	15.25 ± 0.02	15.75 ± 0.05
200	8.78 ± 0.02	6.83± 0.03	11.38 ±0.02	12.62 ±0.02
100	6.17 ±0.03	5.67±0.03	8.02 ±0.01	8.29 ±0.01
50	5.91 ±0.01	-	6.72 ±0.02	6.91 ±0.01
25	-	-	-	-
Cpx (0.05%)	27.50 ±0.01	22.00±0.00	26.70 ±0.01	25.80 ±0.01

Key: - = Not determined; Cpx = Ciprofloxacin; Values are mean ±S.E.M. of triplicate measurements. Unit of extract is in mg/ml.

Table 5: Antibacterial activity of aqueous seed extract of *Garcinia kola*

	Diameter zones of inhibition (mm)			
Extract	*Proteus* sp.	*E.coli*	*Enterobacter* sp.	*Erwinia* sp.
400	7.37 ± 0.03	5.85± 0.02	7.50 ± 0.01	8.00 ± 0.01
200	5.68 ± 0.01	-	5.91 ± 0.01	6.24 ± 0.02
100	-	-	-	5.61 ± 0.01
50	-	-	-	-
25	-	-	-	-
Cpx (0.50%)	27.50 ± 0.04	22.00 ± 0.01	26.70 ±0.01	25.80 ±0.01

Key: - = Not significant; Cpx = Ciprofloxacin; Values are mean ±S.E.M. of triplicate measurements. Unit of extract is in mg/ml.

Table 6: Minimum inhibitory concentration (MIC) of *Garcinia kola* seed extracts

Extract	*Proteus* sp.	*E.coli*	*Enterobacter* sp.	*Erwinia* sp.
EEG	200	400	200	200
AEG	-	-	-	-
CPX	50	100	25	50

Key: – = Not significant; EEG = Ethanolic extract of *Garcinia kola*; AEG= Aqueous extract of *Garcinia kola*; CPX= Ciprofloxacin; Unit of extract is in mg/ml.

Table 8: Minimum bactericidal concentration (MBC) of *Garcinia kola* seed extracts

Extract	*Proteus* sp.	*E.coli*	*Enterobacter* sp.	*Erwinia* sp.
EEG	400	-	400	400
AEG	-	-	-	-
CPX	100	200	50	100

Key: – = Not significant; EEG = Ethanolic extract of *Garcinia kola*; AEG= Aqueous extract of *Garcinia kola*; CPX= Ciprofloxacin; Unit of extract is in mg/ml.

4. Conclusion

The present work has revealed that *G. kola* seed possesses medicinal properties which reside in its phytochemical components. It also demonstrated that extracts obtained from *G. kola* at high concentrations display a good antibacterial activity against the test organisms used, suggesting that they could be used to treat infections caused by the organisms as well as their utilization in traditional medicine.

Conflict of interests: The authors declare that they have no conflict of interests.

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