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ORIGINAL RESEARCH

Antibacterial, Phytochemical and Toxicological Activities of *Garcinia kola* Extracts against Multidrug Resistant Clinical Bacteria

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Abstract

Objective: The rise in multiple antibiotic-resistant microorganisms has led to a decline in the efficacy of many antibiotics, prompting the investigation of alternative solutions such as medicinal plants. To tackle this concerning issue, this study assessed the phytochemical composition, antibacterial activity, and toxicological characteristics of *Garcinia kola* extracts against multidrug-resistant bacteria commonly found in clinical settings.

Material-Method: The cold maceration technique was employed to extract the root and leaf of *Garcinia kola* using water and methanol. The extracts were then subjected to phytochemical screening. Extracts were evaluated for the ability to inhibit the growth of five multi-drug resistant isolates used in this study. An agar well diffusion assay was used to determine the zones of inhibition. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth macro dilution technique. Additionally, brine shrimp lethality assay was conducted to determine the 50% lethal concentration (LC50) of the extracts. Synergistic effects of combination of the extracts for each solvent (root and leaf) were tested against the isolates.

Conclusion: The results showed that the root extract in methanol had the highest yield at 25.58%. All four extracts contained ten out of the fourteen tested phytochemicals. The methanol leaf and root extracts exhibited the highest and lowest inhibition zones of 20 mm and 9 mm against *Staphylococcus aureus*, respectively. The MIC values ranged from 250 to 600 mg/mL indicating broad-spectrum antibacterial activity. The cytotoxicity test showed a range of 33.03 to 126.3 µg/mL for the aqueous and methanol extracts. Although Garcinia kola shows potential as a source of antibacterial compounds, caution should be exercised due to its toxic effects.

Keywords: *Garcinia kola*, Phytochemicals, Minimum Inhibitory Concentration (MIC), Inhibition Zones, Cytotoxicity

INTRODUCTION

Researchers continue to monitor the utilization of botanical substances for addressing various conditions, and traditional herbal remedies have become increasingly significant as alternative treatments for a diverse array of illnesses [1, 2]. The majority of individuals in economically disadvantaged regions perceive plant-based remedies as more cost-effective and safer alternatives [3]. The use of these plant products has expanded in industrialized, emerging, and impoverished nations due to the introduction of new diseases and the development of microorganism resistance [4, 5].

Numerous pharmacologically active compounds present in medicinal plants can improve health through various mechanisms, whether individually, in combination, or both simultaneously [6]. The identification of phytochemicals within plants, which could potentially serve as significant medications in modern medicine, has fueled ongoing interest in evaluating natural compounds derived from plants as potential chemotherapeutic agents [7]. Apart from generating potentially harmful bioactive molecules, plants also develop defense mechanisms against predators [8]. The increasing popularity of medicinal plants has underscored the need for comprehensive scientific investigations to assess both their potential toxicity and effectiveness [9]. While the general public often perceives herbal medicines as safe and devoid of potential toxicity, the risk of toxicity remains a significant barrier that limits their widespread use [10]. Common toxicities associated with herbal medicines include hepatotoxicity, nephrotoxicity, neurotoxicity, cardiac toxicity, pulmonary toxicity, adult respiratory distress syndrome, convulsions, and

acute eosinophilic pneumonia [11]. The exploration of medicinal plant toxicity can be advantageous for both the development of novel therapeutic compounds and the progress of conventional medicine [9].

Many African nations rely on traditional medicine to address their healthcare needs [7]. Furthermore, in the folk medicine of the Ilaje people of Ondo State, Nigeria, appendicitis is treated using the root of the botanical plant *Garcinia kola*. This practice stems from the abundance of secondary metabolites, or phytochemicals, found in plants, which may possess pharmacological properties effective against various ailments [13]. Because of its bitter, astringent taste and stimulating qualities, the seeds are greatly valued as an oral masticatory aid. They, along with other plant components, are utilized as an aphrodisiac and for the treatment of various conditions including diarrhoea, bronchial issues, throat infections, fever, colds, and malaria [14]. In customary, cultural, and social rituals, people traditionally chew the seeds for their aphrodisiac properties [15]. Around 80% of the population in many West African countries resort to medicinal herbs for therapy, primarily due to the limited affordability of new medications [16].

The multifunctional *Garcinia kola* Heckel (Clusiaceae) tree is commonly encountered in tropical and subtropical wet lowland forests across sub-Saharan Africa, including countries like Nigeria and Cameroon. Virtually every part of this tree has been harnessed in traditional medicine for centuries to address a diverse array of ailments. Consequently, it is referred to by various names such as bitter kola, false kola, and occasionally dubbed the "wonder plant" [14]. In recent years, *Garcinia kola* has garnered significant research interest [14, 15, 17, 18] primarily due to its unique biflavonoid complex called kolaviron, which seems to be exclusive to this plant species. However, it is worth noting that *Garcinia kola* contains various other chemicals alongside kolaviron, including garcinianin, kolanone, gakolanone, garcinoic acid, garcinal, garcifuran A and B, and garcipyran [19]. Additionally, these compounds appear to be highly specific to *Garcinia kola*, with no other botanical sources confirming their presence. The objective of this study was to investigate the cytotoxic properties, antimicrobial properties, and phytochemical activities of extracts derived from *Garcinia kola*.

MATERIALS AND METHODS

Plant materials

The plant materials were obtained from a nearby plantation in Shagamu, Ogun State. To confirm the plant's identity, it was examined and authenticated at the herbarium, Department of Botany, University of Ibadan. A voucher specimen labeled UIH-23235 was submitted for future reference. The leaves and roots of the plant were air-dried, pulverized, and soaked in clean distilled water and cold methanol separately. The resulting extracts were concentrated using a vacuum and stored at a temperature of 4°C for later use.

Test organisms and media

The bacterial species [*Staphylococcus aureus, Escherichia coli, Acinetobacter baumannii, Enterobacter cloacae, and Enterococcus faecalis*] used in this study were obtained from the Department of Microbiology, University of Ibadan and University College Hospital, Ibadan, Nigeria. These bacteria were cultured on nutrient agar [No. 2] and nutrient broth [pH 7.4] from Oxoid, England. Prior to testing, all bacteria were kept on agar slopes at 4°C. Brine shrimp eggs [*Artemia salina* Sander®] were purchased from the Great Salt Lake Company in the USA.

Phytochemical screening

Preliminary phytochemical screening was carried out according to Evans [20] and Edeoga *et al.* [21]. An analysis was conducted to determine if the leaves and roots contained certain secondary metabolites. These included alkaloids, tannins, cardiac glycosides, flavonoids, saponins, steroids, phenols, coumarins, terpenoids, diterpenes, flavonoids, anthocyanins, charcones, and proteins.

Cytotoxicity [Brine-Shrimp Lethality] assay

Cytotoxicity test was carried out using the methods of Meyer *et al* [22]. Artemia salina eggs were incubated in seawater for a period ranging from 48 to 72 hours. The nauplii were divided into tubes with different concentrations of extracts and each of the tube contained 10 nauplii. The concentrations used were 1000, 500, 250, 125, 62.5, and 31.25 in µg/mL. After 24 hours, the number of surviving nauplii was recorded to estimate the number of dead nauplii. The mortality percentage for Brine Shrimp nauplii was determined for each concentration using the following formula;

% Mortality = N1/N0 × 100

Where,

N1 = The total count of deceased nauplii after 24 hours of being kept at room temperature.

N0 = Total number of nauplii

The Probit analysis was conducted using the conventional probit table. Using this information, we determined the median Lethal Concentration [LC_{50}] following the methodology outlined by Finney [23].

Toxicity testing criteria

Herbal extract toxicity is often evaluated using either Meyer's or Clarkson's toxicity index. In line with Meyer's index, extracts are considered toxic if the LC_{50} value is below 1000 µg/mL, and non-toxic if the LC_{50} value is above 1000 µg/mL[22]. On the other hand, Clarkson's toxicity criterion categorizes plant extracts as follows: non-toxic for LC_{50} values above 1000 µg/mL; low toxic for LC_{50} values between 500 and 1000 µg/ml; medium toxic for LC_{50} values between 100 and 500 µg/mL, and highly toxic for LC_{50} values between 0 and 100 µg/mL[24].

Determination of antibacterial activity

Antibacterial activity of the extracts was determined by agar well diffusion method of Perez et al. [25], with slight modifications Idowu et al. [26]. The samples were dissolved in sterile distilled water and methanol to achieve concentrations ranging from 125-750 mg/mL. Each test organism was sub-cultured in Nutrient broth and incubated for 24 hours and adjusted using the 0.5 McFarland standard. A sterile cotton swab was placed into the prepared liquid and rotated a few times by applying pressure to the inside of the tube. This action was done to remove any extra liquid from the swab. Next, the swab was inoculated onto the Mueller Hinton Agar (MHA) plate by moving it back and forth across the whole surface. The plate was also rotated about 60° after each streak to ensure the inoculum was evenly spread. Afterward, the MHA plates remained uncovered for three to five minutes to enable the absorption of any additional moisture present on their surfaces (CLSI) [27]. An 8.0 mm cork borer was utilized to create holes in agar, and then 100µL of extracts were added to each well. Controls were established using a concentration of 15µg/mL of Erythromycin for the bacteria, while 40% methanol served as the negative control. The size of the inhibition zones was employed as an indicator of the antibacterial effectiveness.

Determination of minimum inhibitory concentration [MIC]

Minimum Inhibitory Concentration [MIC] was determined on test bacteria by broth macro dilution using the method of Andrews [28]. The agar was combined with the extracts to create a range of dilutions in each test tube, including concentrations of 600, 500, 400, 300, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, and 1.95 mg/mL. All test tubes contained 2 mL of nutrient broth, and then 0.1 mL of each extract at the desired concentration was added to the broth. Next, 0.1 mL of standardized inoculums of the specific test pathogens were introduced into the test tubes that contained both the nutrient broth and the extract suspensions. All the test tubes were incubated at 37°C for 24 hours. Following the incubation period, each test tube was examined to determine whether visible signs of bacterial growth were present or absent. The minimum concentration of the substance being tested, at which no observable bacterial growth was detected, was defined as the MIC [Minimum Inhibitory Concentration].

Determination of minimum bactericidal concentration [MBC]

To determine the minimum bactericidal concentration [MBC] of an extract, a loop full from each broth culture that did not show any growth in the minimum inhibitory concentration [MIC] tubes was inoculated onto freshly prepared nutrient agar plates and incubated for 24 hours. At the end of the incubation period, the extract with lowest concentration that prevented any bacterial growth on the solid medium was considered as the MBC for the extract. This observation was associated with the MIC test tube that exhibited no signs of growth within 24 hours of incubation.

RESULTS

Table 1 shows the percentage of plant extract obtained from the different solvents. The root extracted using methanol demonstrated the highest yield of 22.60%, whereas the aqueous root extraction was the lowest with 4.24%.

The composition of phytochemicals in *Garcinia kola* extracts from the leaf and root are presented in Table 2. The results demonstrated that both the root and leaf samples of *Garcinia kola* contain various phytochemicals of interest, including alkaloids, tannins, flavonoids, saponins, phenols, and quinones. The quantitative analysis of phytochemicals indicates that ten out of the fifteen tested bio-active constituents were found in both the methanolic and aqueous extracts of *Garcinia kola*. However, charcones, anthocyanins, and chalcones were not detected. Coumarins were only absent in the aqueous root extract, and cardiac glycosides were only present in the aqueous root extract.

Table 1. Percentage yield of *Garcinia kola* extracts

Plant parts	Solvent Type	Weight of sample [g]	Weight of extracts [g]	Yield [%]
Leaves	Aqueous	1000	66.30	6.63
Leaves	Methanol	1000	168.00	16.80
Root	Aqueous	1000	42.40	4.24
Root	Methanol	1000	226.00	22.60

Table 2. Qualitative phytochemical Screening of *Garcinia kola* Extract

S/N	Phytochemicals	Extracts			
		Aqueous Root	Methanol Root	Aqueous Leaf	Methanol Leaf
1	Saponins	+	+	+	+
2	Tannins	+	+	+	+
3	Flavonoids	+	+	+	+
4	Steroids	+	+	+	+
5	Quinones	+	+	+	+
6	Terpenoids	+	+	+	+
7	Phenols	+	+	+	+
8	Di-terpenes	+	+	+	+
9	Proteins	+	+	+	+
10	Alkaloids	+	+	+	+
11	Cardiac glycosides	+	-	-	-
12	Coumarins	-	+	+	+
13	Chalcones	-	-	-	-
14	Anthocyanins	-	-	-	-

Table 3 displays the results of our findings on the phytochemical screening of the *Garcinia kola* extracts. The table presents the percentage of active compounds found in the aqueous and methanol extracts. It was observed that the root and leaf extracts from methanol had higher levels of active compounds compared to the aqueous root and leaf extracts. Specifically, the methanol extract of the root exhibited the highest concentration of phenols [83.501±0.053], while the aqueous extract of the root had the lowest concentration of saponins [0.60±0.10%].

Table 3. Quantitative phytochemical screening of *Garcinia kola* extracts

Sample	Saponins [%]	Alkaloids [%]	Flavonoids [mg QE/g]	Phenols [mg GAE/g]	Tannins [mg GAE/g]
Methanol Root	4.28±0.02	12.14±0.06	42.889±0.00	83.501±0.05	10.608±0.05
Methanol Leaf	9.50±0.01	10.40±0.00	29.630±0.13	31.795±0.03	5.474±0.04
Aqueous Root	4.61±0.02	3.91±0.01	10.963±0.78	26.472±0.05	4.982±0.00
Aqueous Leaf	0.60±0.10	1.82±0.02	3.778±0.00	4.899±0.03	3.123±0.04

Values are in mean of duplicate values ± Standard error

Table 4 presents the results of the study on the antibacterial properties of aqueous leaf and root extracts of *Garcinia kola* measuring the zones of inhibition, in millimeters, of the extracts against different bacteria. The results revealed that the extracts displayed varying levels of antibacterial activity against the test bacteria. In general, the root extracts exhibited higher inhibition zones than the leaf extracts, particularly against *Staphylococcus aureus, Escherichia coli,* and *Enterococcus faecalis.* Notably, the root extracts were particularly effective against *Enterococcus faecalis*, with a zone of inhibition of 13.50±0.71 mm. However, the aqueous extracts exhibited no antibacterial activity against *Acinetobacter baumannii* and *Enterobacter cloacae*. Table 5 shows the antibacterial activity of methanol leaf and root extracts of *Garcinia kola* depicted by zone inhibition of the isolates in millimeters. Results also showed variations in the antibacterial activities of the extracts against the test bacteria. The zones of inhibitions of the methanol extracts were generally higher than those of the aqueous extracts. The extracts were very effective against three of the test isolates [*S. aureus, E. coli, E. faecalis*], although more effective on *Staphylococcus aureus* [20.00±0.00mm] than the other two isolates. The methanol extracts showed no antibacterial activity on *A. baumannii* and *E. cloacae*.

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Table 4. Antibacterial activity of Aqueous Leaf and Root Extracts of *Garcinia kola*

Extracts	Conc. In mg/mL	*Escherichia coli*	*Acinetobacter baumannii*	*Staphylococcus aureus*	*Enterococcus faecalis*	*Enterobacter cloacae*
AR	750	13.00±2.83	0.00±0.00	12.00±1.41	13.50±0.71	0.00±0.00
	500	11.00±1.41	0.00±0.00	12.00±0.00	11.00±0.00	0.00±0.00
	250	9.00±1.41	0.00±0.00	11.50±0.71	11.00±0.00	0.00±0.00
	125	9.00±0.00	0.00±0.00	9.00±0.00	9.50±0.71	0.00±0.00
AL	750	10.50±0.71	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	500	11.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	250	9.50±0.71	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	125	9.50±0.71	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
ARAL	750	13.50±0.71	0.00±0.00	14.50±0.71	12.50±0.71	0.00±0.00
	500	12.00±0.00	0.00±0.00	14.00±0.00	10.50±0.71	0.00±0.00
	250	11.00±0.00	0.00±0.00	13.50±0.71	10.50±0.71	0.00±0.00
	125	11.00±0.00	0.00±0.00	12.50±0.71	9.00±0.00	0.00±0.00
Control	Erythromycin [15µg]	0.00±0.00	8.00±0.00	0.00±0.00	10.00±0.00	7.00±0.00
	Methanol [40% v/v]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

AR: Aqueous Root AL: Aqueous Leaf ARAL: Aqueous Root and Aqueous Leaf

Table 5. Antibacterial activity of Methanol Leaf and Root Extracts of *Garcinia kola*

Extracts	Conc. in mg/mL	*Escherichia coli*	*Acinetobacter baumannii*	*Staphylococcus aureus*	*Enterococcus faecalis*	*Enterobacter cloacae*
MR	750	12.50±0.71	0.00±0.00	14.50±0.71	12.00±0.00	0.00±0.00
	500	13.00±0.00	0.00±0.00	13.00±1.41	14.00±0.00	0.00±0.00
	250	12.50±0.71	0.00±0.00	12.50±2.12	13.00±1.41	0.00±0.00
	125	11.00±0.00	0.00±0.00	11.00±0.00	15.00±0.00	0.00±0.00
ML	750	12.50±0.71	0.00±0.00	19.00±0.00	12.00±1.41	0.00±0.00
	500	13.00±0.00	0.00±0.00	17.50±0.71	12.00±1.41	0.00±0.00
	250	12.50±0.71	0.00±0.00	17.50±0.71	18.50±0.71	0.00±0.00
	125	11.00±0.00	0.00±0.00	15.00±0.00	17.00±0.00	0.00±0.00
MLMR	750	12.00±0.00	0.00±0.00	15.00±0.00	15.00±0.00	0.00±0.00
	500	9.50±0.71	0.00±0.00	20.00±0.00	14.50±0.71	0.00±0.00
	250	11.00±0.00	0.00±0.00	17.00±0.00	9.00±0.00	0.00±0.00
	125	12.00±0.00	0.00±0.00	13.00±2.82	14.50±0.71	0.00±0.00
Control	Erythromycin [15µg]	0.00±0.00	8.00±0.00	0.00±0.00	10.00±0.00	7.00±0.00
	Methanol [40% v/v]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

MR: Methanol Root ML: Methanol Leaf MLMR: Methanol Root and Methanol Leaf

Tables 6 and 7 show the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the aqueous and methanol extracts [Root and Leaf] of *Garcinia kola*. Results showed the MIC values to be within the range of 125 – 600 mg/mL.

Table 8 showed the LC_{50} as calculated by Graph Pad Prism 2021 (Computer software programme) to be within the range of 33.03-126.3 µg/mL. Table 9 shows Finney's table for transformation of percentage of mortality to probit values.

Table 6. Minimum Inhibitory Concentration [MIC] and Minimum Bactericidal Concentration of the aqueous extract [Root and Leaf] of *Garcinia kola*

EXTRACT	MG/ML	*Escherichia coli*	*Acinetobacter baumannii*	*Staphylococcus aureus*	*Enterococcus faecalis*	*Enterobacter cloacae*	Sterility
AR	MIC	300	>600	300	300	>600	----
	MBC	600	>600	>600	500	>600	NG
AL	MIC	300	>600	250	250	>600	----
	MBC	>600	>600	>600	>600	>600	NG
ALAR	MIC	600	>600	125	400	>600	----
	MBC	600	>600	400	>600	>600	NG
Control	EI	NG	+	NG	+	+	NA
	MI	+	+	+	+	+	NA
	BI	+++	+++	+++	+++	+++	NA

AR: Aqueous Root ---: No turbidity ARAL: Aqueous Root and Aqueous Leaf +++: Massive Growth
AL: Aqueous Leaf NG: No growth NA: Not applicable +: Growth
EI: Erythromycin + Inoculum MI: Methanol + Inoculum BI: Mueller Hinton Broth + Inoculum

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Table 7. Minimum Inhibitory Concentration [MIC] and Minimum Bactericidal Concentration of Methanol extract [Root and Leaf] of *Garcinia kola*

EXTRACT	MG/ML	*Escherichia coli*	*Acinetobacter baumannii*	*Staphylococcus aureus*	*Enterococcus faecalis*	*Enterobacter cloacae*	Sterility
MR	MIC	300	>600	250	250	>600	----
	MBC	>600	>600	400	400	>600	NG
ML	MIC	400	>600	300	300	>600	----
	MBC	>600	>600	500	>600	>600	NG
MLMR	MIC	400	>600	300	400	>600	----
	MBC	600	>600	500	>600	>600	NG
Control	EI	NG	+	NG	+	+	NA
	MI	+	+	+	+	+	NA
	BI	+++	+++	+++	+++	+++	NA

MR: Methanol Root ---: No turbidity ML: Methanol Leaf NG: No growth MLMR : Methanol Root and Methanol Leaf
NA: Not applicable +++: Massive Growth +: Growth
EI: Erythromycin + Inoculum MI: Methanol + Inoculum BI: Mueller Hinton Broth + Inoculum

Table 8. Toxicity of Aqueous and Methanol Extracts of *Garcinia kola* [Root and Leaf] using Brine Shrimp Lethality Assay

Extract	Concentration [µg/mL]	No. of 1st tube	Survived 2nd tube	nauplii 3rd tube	No. of dead nauplii	% Mortality	Probit	LC_{50}
AR	1000	0	0	0	30	100.00	8.09	33.03
	500	0	0	0	30	100.00	8.09	
	250	0	1	2	27	90.00	6.28	
	125	1	2	2	25	83.33	5.95	
	62.5	3	3	4	20	66.66	5.41	
	31.25	4	5	6	15	50.00	5.00	
AL	1000	0	0	0	0	100.00	8.09	76.06
	500	0	0	0	0	100.00	8.09	
	250	0	0	0	0	100.00	8.09	
	125	4	2	3	4	70.00	5.52	
	62.5	5	5	6	5	46.66	4.90	
	31.25	8	9	8	8	16.66	4.01	
MR	1000	0	0	0	30	100.00	8.09	84.29
	500	0	0	0	30	100.00	8.09	
	250	2	2	3	23	76.67	5.71	
	125	3	4	4	19	63.33	5.33	
	62.5	4	6	5	15	50.00	5.00	
	31.25	7	8	8	7	23.33	4.26	
ML	1000	0	0	0	30	100.00	8.09	126.30
	500	0	0	0	30	100.00	8.09	
	250	0	0	0	30	100.00	5.71	
	125	4	6	6	14	46.67	5.33	
	62.5	8	7	8	7	23.33	5.00	
	31.25	10	9	10	1	3.33	4.26	
CYCLOPHOSPHAMIDE	1000	2	2	2	24	80	5.84	61.82
	500	3	3	4	20	66.67	6.41	
	250	4	3	4	19	63.33	5.33	
	125	5	4	6	15	50	5.00	
	62.5	7	7	6	10	33.33	4.56	
	31.25	7	9	9	5	16.67	4.01	

AR= Aqueous Root; AL= Aqueous Leaf; MR= Methanol Root; ML= Methanol Leaf

Table 9. Finney's table for transformation of percentage of mortality to probit values

%	0	1	2	3	4	5	6	7	8	9
0	__	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.25	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
__	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

(Finney, 1952).

DISCUSSION

In response to the increasing problem of drug-resistant bacteria, there is an ongoing need to research new treatments to tackle the global issue of antimicrobial resistance. The World Health Organization firmly advocates for the exploration of traditional medicines as potential sources of safe and effective remedies, not only for microbial infections but also for non-microbial illnesses (WHO) [29]. This research explored the potential medicinal properties of *Garcinia kola* extract, an indigenous plant, and its ability to effectively treat drug-resistant bacteria responsible for wound and urinary tract infections often encountered in healthcare settings. It equally replicated the use of traditional folk medicine practices that have been proven effective in treating infections over time. In ancient times, the use of medicinal plants provided significant benefits in managing infectious diseases. Therefore, it is important to follow a similar approach as closely as possible to the traditional methods used in those historical periods. In this study, a cold maceration method was used, which is in line with traditional practices, as it enables a gentle extraction process. This method was observed to effectively preserve most of the phytoconstituents found in the plant materials. The differences observed in the percentage yield, as illustrated in Table 1, can be ascribed to the utilization of various plant components and solvents as opined by Heinrich *et al.* [30]. Both methanol root and methanol leaf had the highest percentage of yield coming in with 22.6% and 16.80%, respectively, and this might be due to the high volatile property of methanol as a solvent which is able to extract both polar and non-polar compounds.

Qualitative phytochemical screening of *Garcinia kola* extracts revealed the presence of tannins, saponins, flavonoids, alkaloids, steroids, quinones, terpenes, di-terpenes, phenolic compounds in all test extracts, and this is in accord with the work of Ukaoma *et al.* [31] who equally detected four of these compounds (Flavonoids, tannins, alkaloids and saponins) in their study. In the study of Emmanuel *et al.* [32] on *G. kola* roots, bioactive compounds such as saponins, tannins, flavonoids, cardiac glycosides, and alkaloids were also identified; however, it is noteworthy that the researchers did not utilize methanol as a solvent in their study. This indicates that these key bioactive components are naturally found in the *G. kola* plant, regardless of the choice of solvent for extraction. Flavonoids are well-known for their capacity to defend the body against damaging molecules like hydroxyl and superoxide anion radicals, which ultimately contribute to promoting overall health [32]. Flavonoids possess various beneficial properties, such as anti-inflammatory, anti-allergic, analgesic, and antioxidant effects; this backs the traditional usage of *Garcinia kola* in folk medicine to treat various infections [33]. Tannins have been identified as a phytochemical compound in *Garcinia kola* extract; these compounds have antimicrobial properties and can inhibit the growth and multiplication of microorganisms by binding to iron, forming hydrogen bonds, and interacting with important proteins in these organisms [34]. Presence of tannins makes *Garcinia kola* a suitable medicinal plant for treating microbial infections, especially gastrointestinal ailments like diarrhoea and dysentery [35]. Additionally, tannins have shown promise in preventing and acting as anticancer agents, making them valuable compounds in cancer research [36]. Various factors, both prior to and during the extraction process, can impact the extraction of phytochemicals. These factors encompass the specific part of the plant utilized, the locality and particle size, the drying technique employed, fluctuations in daily and seasonal conditions, and the degree of processing, among others. Additionally, factors associated with the extraction process itself, such as the chosen extraction technique, solvent type, ratio of solvent to sample, solvent pH and temperature, and extraction duration, can also influence the extraction process. In summary, these diverse factors can shape the results and render different variations in the extraction process feasible [37].

The results of the quantitative phytochemical analysis in Table 3 indicate that both solvents (methanol and water) were effective in extracting active compounds from the plant. Methanol was more effective in extracting certain active compounds, particularly flavonoids [42.889±0.000] and phenols [83.501±0.053]. The high efficiency observed in methanol solvent may be due to its high volatile nature. Methanol-extracted root had the highest content in four out of five of the tested phytochemicals, which can be attributed to high polyphenol content commonly found in *G. kola* root. This is consistent with the presence of bioflavonoids in the seeds, stem bark, and roots of *Garcinia kola* [38,39].

In the antibacterial activity, extracts from the root [Table 4] largely had higher inhibition zones on three of the test isolates [*S. aureus, E. coli, E. faecalis*], although it was more effective on *Enterococcus faecalis* [13.50±0.71 mm] than other isolates. The aqueous extracts showed no antibacterial activity on *A. baumannii* and *E. cloacae*. These findings showed that *G. kola* can be used comprehensively in the treatment of bacteria diseases, mainly the root extract [40].

The combination of the aqueous root and aqueous leaf extracts (ALAR) (1:1), as shown in Table 4, showed a higher antibacterial activity across all four concentrations against the test bacteria than when it was tested singly, and the highest antibacterial activity was against *S. aureus* with a zone of inhibition of 14.50mm at 750 mg/mL. These results indicate that there may be a synergistic effect when these extracts are combined, resulting in enhanced effectiveness. This could be because the compounds in the extracts collaborate to target various aspects of bacterial physiology, resulting in a more significant overall impact compared to using the compounds individually. Furthermore, both the methanolic and aqueous extracts of *Garcinia kola* demonstrated inhibition of the growth of both Gram-positive and Gram-negative bacteria. The use of methanol in the extraction process resulted in a stronger inhibitory effect compared to the aqueous extract, possibly due to the higher concentration of active compounds in the methanol extract. The results of this study indicate that there is a range of antibacterial activities among the different extracts, which is likely attributed to variations in the quantity of compounds present in each plant extract. Phenols [83.501±0.053 mg GAE/g] from methanol extraction were the highest observed and this can be attributed to the high volatility of methanol, which led to more active ingredients than in water. The lethal effect, as observed in the results, was higher in *Staphylococcus aureus* [20.00 mm]. No antibacterial activity for both *A. baumannii* and *E. cloacae* clearly shows resistance of both isolates to the *Garcinia kola* root and leaf extracts, and this could be due to several reasons. Both bacteria are opportunistic pathogens and are often associated with nosocomial infections and can be difficult to treat due to their high antibacterial resistance. The bacteria being resistant to the extracts even at high concentrations suggests that the active compounds in the extracts are ineffective against the strains. It is possible that these bacteria have developed resistance mechanisms that allows them to resist the action of the antimicrobial compounds present in the extracts. Reports on the antimicrobial activities of *G. kola* vary considerably as there are studies with different views and ranges of antibacterial action [41, 42, 43]. However, it is noteworthy to mention that different parts of the plant were used.

In this study, it was noticed that the isolates resistant to multiple antibiotics displayed higher minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values, which varied among the different bacteria strains. When the extracts were used at concentrations ranging from 1.95-62.5mg/mL, it was observed that they were not effective in inhibiting bacterial growth. However, at higher concentrations (125-600mg/mL), we observed varying inhibitory and lethal concentrations for different bacteria. Interestingly, both *A. baumannii* and *E. cloacae* showed no MIC or MBC values, indicating that none of the extracts was able to inhibit or kill these bacteria even at the highest concentration tested (600mg/mL). This lack of activity may be attributed to the factors mentioned earlier in the study. It is worth noting that unlike the agar well diffusion assay, there was no evidence of synergy in the MIC assay. In the study of Osungunna [44], it was suggested that the extracts may have shown a combined effect in the agar well diffusion assay, but not in the MIC test, possibly because of variations in the experimental settings. The agar well diffusion assay is a qualitative technique that can detect even trace amounts of extracts, whereas the MIC test is a quantitative approach that determines the lowest concentration needed to hinder microbial growth. Aqil and Ahmad [45] suggested that the differences in the results of the two tests could be attributed to various factors. One possibility is that the microorganisms in the two tests may have had different susceptibility patterns, which could impact the outcomes. Additionally, variations in the preparation and dilution of the substances being tested, as well as the incubation conditions, could also contribute to disparities in the results between the two tests. The significant variations in the values of MIC and MBC observed in this study may be due to the bacteria's ability to develop resistance to multiple antibiotics. Furthermore, the diverse types of plant components and their combined effects, along with the inherent resistance of each multi-drug resistant bacterium, may also play a role in

these differences, as different bacteria may react differently to the test samples. Thormar [46] suggested that researchers should prioritize antimicrobial agents with MIC values lower than 1% vol/vol (equivalent to 10,000 ppm) in laboratory settings. This means these agents can effectively hinder or eliminate bacteria at low concentrations. It is essential to keep in mind that findings from laboratory experiments may not accurately represent real-life situations within the body. *In vivo* conditions involve varying concentrations of antibacterial agents and bacteria throughout different bodily regions, and these values are not considered fixed constants [44].

In contemporary times, the brine shrimp (*Artemia salina*) lethality assay has become a frequently employed method to assess the potential harmful impact of bioactive substances. This assay serves as an initial step in evaluating the toxicity of plant extracts for screening purposes. [47,48,49]; pesticides [50] and nanostructures [51]. The initial proposal for this assay was made by Michael *et al.* [50], and it was further developed by other researchers. This lethality assay has been used as a guide for evaluating the cytotoxic and antitumor properties of active agents, as demonstrated by Meyer *et al.* [22] in 1982. In general, it is commonly believed that extracts obtained using alcohol or organic solvents tend to be more toxic than those obtained using water. However, our study yielded contrasting results, as we discovered that the aqueous extracts were actually more toxic than the methanol extracts. Our research findings indicate that the aqueous root, aqueous leaf, and methanol root extracts of *Garcinia kola* are not safe and exhibit toxicity towards the nauplii, with LC_{50} values of 33.03, 78.06, and 84.09 µg/ml, respectively. Additionally, the methanol leaf extract displayed medium toxicity towards the nauplii, with an LC_{50} value of 126.3 µg/ml, in accordance with Clarkson et al. [24] findings. This report corroborates the study of Onajobi et al [52] where they also found that the aqueous extract of *Garcinia kola* was more toxic than the methanol extract. The authors suggested that the higher toxicity of the aqueous extract could be due to the higher solubility of some toxic compounds in water compared to methanol.

CONCLUSION

The findings of this study emphasize the promising potential of *Garcinia kola* as a valuable treatment option for infectious diseases caused by strains that are resistant to multiple antibiotics. The *Garcinia kola* plant showed effective activity against bacteria used in this study and could be further studied for use in management of infections when compared to conventional antibiotics. These findings offer a scientific foundation to consider utilizing the plant for medicinal purposes in treating various infectious diseases such as skin infections, wound infections, and urinary tract infections. Although the results have demonstrated the antibacterial properties of this medicinal plant, further research and development are necessary before the plant can gain broader recognition among the general population. However, it is important to note that further extensive and thorough toxicological assessments are required to establish the safety of these extracts before they can be confidently used.

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