

ANTIBACTERIAL ACTIVITY OF GARCINIA KOLA SEED AND LEAF EXTRACT ON SOME SELECTED CLINICAL ISOLATES

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ABSTRACT

Garcinia kola seeds and leaves were screened for their anti bacterial activities using 1% hydrochloric acid, diethylether, acetic acid (ethanoic acid) and acetone. The seeds and leaves were dried and ground into powdered form. 10g of the ground seeds and leaves were in four sets. 90ml of the extraction solvents was added to each set. The seed and leaf extracts were tested on clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Streptococcus pyogenes* using Agar Diffusion Method. Acetic acid seed and leaf extract showed the highest degree of zones of inhibition of 44mm and 37mm respectively against all the test organisms. The other extracts showed no inhibitory effect on the test organisms. The mechanism of action of the seed and leaf extract on the four bacteria had leakage of both protein and potassium (K⁺) ions. Phytochemical screening of the extracts revealed the presence of some bioactive components like alkaloids, saponins, tannins, anthraquinones and cardiac glycosides. These components determine the antibacterial activity of the seed and leaf extracts. The results from this study, provides scientific evidence that *Garcinia kola* has the capability of inhibiting the growth of pathogenic micro organisms; thus it will be useful in tropical medicine for the treatment of microbial infections.

KEYWORDS: *Garcinia kola*, Clinical isolates, Zone of inhibition, Protein and potassium ions leakage, Phytochemical screening

INTRODUCTION

Garcinia kola, also known as bitter kola, is a nut-bearing tropical tree native to Nigeria's coastal rainforests. It was named after a man called Garcin, who lived and wrote about the plant in the 18th century [1]. It has a bitter taste followed by slight sweetness. Despite its bitter taste, *Garcinia kola* nuts are commonly eaten as a snack and used for their stimulant effects, due to high caffeine content. *G.kola* is mostly found in the southern part of Nigeria [2].

Garcinia kola contains antibacterial properties, according to clinical data. They are effective in the treatment of infectious disease while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [3]. They are effective, yet gentle. They exhibit response to specific organs or systems in the body.

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Bitter kola does not possess toxins. In the United States, bitter kola is termed as "miracle drug" [4].

G.kola has been shown to be a popular treatment for diarrhea and fever. The seed extract is antiseptic and is active mostly against gram positive bacteria. While the leaf, is active mostly against gram negative bacteria [5]. It is also very efficacious for hepatitis. In West Africa, is now being harnessed as a cure for the Ebola virus infections and also against flu [3]. The stem, bark and the seeds are used for acute fever, inflammation of the respiratory tract and throat infections. Historically, Nigerians used *Garcinia kola* as an aphrodisiac. The seeds are also chewed to relieve hoarseness of voice, sore throat and cough. In folk medicine the seed is used for the treatment of liver disorder [6]. It is also used in the treatment of dysentery and diarrhea [7]. The leaves are used for stomach ache and pains and is also anti helminthic. They also serve as good remedy for typhoid fever [8].

The seed and leaf of bitter kola are complex mixture of biflavonoids and prenylated benzophenones [9]. They have anti-inflammatory, anti-diabetic and anti-heptotoxic properties. There are many pharmacological effects demonstrated for *Garcinia* biflavanoids [10]. Recently, two new chromanols, garcinnoic acid, garcinial, together with 8-tocotrienol were reported. *G.kola* also contains tannins, cardiac glycosides, saponins, alkaloids, hydroxymethyl anthraquinones, phlobatannins, polyphenols, glucosides and reducing compounds [11].

THE OBJECTIVE OF THE STUDY

The objective of this study was to determine the antibacterial effect of the seed and leaf extracts of *Garcinia kola* on clinical isolates of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi* and *Escherichia coli*.

MATERIALS AND METHODS

Sample collection

Seeds of *Garcinia kola* were collected locally from New Benin market in Benin City, Edo State, Nigeria while pesticide free *Garcinia kola* leaves, collected in Igbu village, Ekpoma, Edo State, Nigeria.

Collection of Test Organisms

Properly identified clinical isolates of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Salmonella typhi* were collected from the Microbiology Laboratory Department of Faith Medical Centre Benin City, Nigeria. The isolates were re-identified using conventional microbiological techniques [12].

Preparation of Extracts

100g of fresh leaves and seeds were each dried in hot-air oven at 50°C for 4 days. The leaves and seeds were then ground into powdery form. Ten grams (10g) each of the grounded seed and leaf were weighed into 100ml sterile bottles. 90ml of each solvent (1% hydrochloric acid, diethylether, acetic acid (ethanoic acid) and acetone) was dispensed into the sterile bottles. The bottles were placed on a mechanical shaker overnight for extraction. The mixture was filtered into sterile 100ml bottles with the aid of Wattman no 1 filter paper. This was done for each extract.

Sterility test of the Extracts

Purity of extracts was determined by streak-inoculation onto freshly prepared Nutrient, Blood and MacConkey agar plates. Plates were incubated

at 37°C for 18-24 hours. At the end of incubation period, plates were examined for growth.

Serial Dilution of Extracts

1% Hydrochloric acid, Acetone, Acetic acid and Diethylether were the solvents used for serial dilution of the extracts. Different ratios of the extract to solvent were obtained. The control for the experiment contained 1ml of the solvent hence apportioned 0:1

The 2nd set of dilution contained 1ml of the different extracts only that is (1:0).

The 3rd set of dilution contained 1ml of extract mixed with 2ml of solvent (1:2).

The 4th set of dilution contained 1ml of extract mixed with 4ml of solvent (1:4).

The 5th set of dilution contained 1ml of extract mixed with 6ml of solvent (1:6)

The 6th set of dilution contained 1ml of extract mixed with 8ml of solvent (1:8).

Estimation of Extract Activity using Agar Diffusion Method

A 10-fold serial dilution of overnight broth culture of test organisms was seeded onto the surface of freshly prepared Nutrient agar and labeled filter paper discs impregnated with each extract for 4 hours was placed on the surface of the Nutrient agar plates using a sterile forceps. Quantification of microbial growth inhibition was determined by measuring the diameter of clear zones around the disc after 24 hours of incubation.

Determination of Extract Antimicrobial Activity

For the determination of the extract minimum inhibitory concentration (MIC), 10-fold serial dilution of each of the different test organism (*Staphylococcus aureus*, *Streptococcus pyogenes* and *Salmonella typhi* and *Escherichia coli*) was carried out. 0.5ml of nutrient broth and 0.5ml of the diluted test organism were dispensed into several tubes after which 1ml of the extract was used to carry out 2-fold serial dilution. After which all the test tubes were incubated at 27°C for 18-24hours. At the end of the incubation period, the test tubes were observed for turbidity (growth).

Determination of Mechanism of Action of Crude Extract

This was done to determine the mechanism of action of the crude extract on the test organisms. Exactly 1ml of 18hours broth of the test organisms was introduced into 30mg/ml concentration of both the seed and leaf crude extract of *Garcinia kola*. The suspension was thoroughly mixed at room temperature (25°C -28°C) and then centrifuged at different intervals of 30minutes, 1hour, 1hour 30minutes and 2 hours. The protein leakage was known by measuring the concentration of protein in the supernatant. This was achieved by reading the absorbance using the flame colourimeter. The corresponding protein concentration was known from a standard curve obtained using Bovine Serum Albumin(BSA).

For the potassium ion leakage, extractions were made at different time intervals 10minutes, 20minutes, 40minutes and 60minutes. The atomic absorption spectrophotometer was used to get the readings. All samples prepared for reading were well centrifuged and the supernatants were used for analysis. Control experiment was carried out with extracts alone with no organisms introduced.

Phytochemical Screening of the Extracts

The extracts obtained were subjected to phytochemical screening by methods of Harbone, 1984 [13]; to determine the presence of bioactive agents such as alkaloids, saponins, tannin, phlobatannins, anthraquinones and cardiac glycosides.

RESULTS

No bacteria growth was observed in all extracts when plated on nutrient agar, blood agar and macConkey agar plates. The zone of inhibition by acetic acid seed extract of *G.kola* on *Staphylococcus aureus* *Streptococcus pyogenes* and *Salmonella typhi* and *Escherichia coli* is shown in table 1.

The serial dilution of both the seed and leaf acetic acid extract resulted in varying diameters of zone of inhibition of the test organisms. For the acetic acid seed extract, the minimum inhibitory concentration for the gram positive and gram negative bacteria was 1:0 and 1:6 respectively (table 1). For the acetic acid leaf extract, the minimum inhibitory

concentration for the gram positive and gram negative bacteria was 1:0 and 1:4 respectively (table 2).

The test organisms were not susceptible to the other extracts such as Acetone, Diethylether and 1% Hydrochloric acid extract of both the seeds and leaves. The maximum dilution at which no growth occurred in the test-tubes was recorded as the minimum inhibitory concentration. For the Gram negative bacteria, the MIC was the seventh test tube (2^{-7}) of the acetic acid leaf extract; the test-tube had a clear content. While for gram- positive bacteria the MIC was the eighth test tube (2^{-8}) for the leaf acetic acid extract. For the acetic acid seed extract, the MIC for gram negative bacteria was the fourth test tube (2^{-4}) while for gram positive bacteria was the third test tube (2^{-3}). The control showed some degree of turbidity.

Table 3-7 shows the values obtained for potassium (K^+) ion and protein leakage from the test organisms by both the seed and leaf extracts of *Garcinia kola*. Table 8 shows the presence of various bioactive components in the extracts. Table 9 shows the diameter of zones of inhibition of the test organisms to some commercial antibiotics.

Table 1: Zone of inhibition by acetic acid seed extract of *G.kola* on *Staphylococcus aureus* *Streptococcus pyogenes*, *Salmonella typhi* and *Escherichia coli*

ORGANISMS	RATIO OF THE EXTRACT TO SOLVENT	DIAMETER OF ZONE OF INHIBITION(mm)
<i>Staphylococcus aureus</i>	0:1	20.0
	1:0	13.0
	1:2	44.0
	1:4	26.0
	1:6	35.0
	1:8	29.0
<i>Escherichia coli</i>	0:1	11.0
	1:0	16.0
	1:2	16.0
	1:4	22.0
	1:6	22.0
	1:8	35.0
<i>Streptococcus pyogenes</i>	0:1	6.50
	1:0	14.0
	1:2	23.0
	1:4	14.0

	1:6	18.5
	1:8	16.5
<i>Salmonella typhi</i>	0:1	4.5
	1:0	9.0
	1:2	9.0
	1:4	16.5
	1:6	12.5
	1:8	19.5

Table 2: Zone of inhibition by acetic acid leaf extract of *G.kola* on *Staphylococcus aureus* *Streptococcus pyogenes*, *Salmonella typhi* and *Escherichia coli*

ORGANISMS	RATIO OF THE EXTRACT TO SOLVENT	DIAMETER OF ZONE OF INHIBITION(mm)
<i>Staphylococcus aureus</i>	0:1	11.0
	1:0	15.0
	1:2	16.0
	1:4	20.0
	1:6	17.5
	1:8	15.0
<i>Escherichia coli</i>	0:1	6.0
	1:0	4.0
	1:2	8.0
	1:4	10.0
	1:6	17.5
	1:8	18.5
<i>Streptococcus pyogenes</i>	0:1	11.0
	1:0	28.0
	1:2	17.0
	1:4	24.0
	1:6	35.0
	1:8	26.0
<i>Salmonella typhi</i>	0:1	8.0
	1:0	11.0
	1:2	12.0
	1:4	18.0
	1:6	35.0
	1:8	37.0

Table 3: Biuret Method of Protein Determination used as the standard for the determination of protein leakage

Standard at Bovine Albumin(ml)	BSA-Serum Concentration of BSA used	Water (mg)	Biuret Reagent(ml)	Absorbance 540mm
0.5	1	3.5	2	0.001
1.0	2	5.0	2	0.02
1.5	3	2.5	2	0.02
2.0	4	2.0	2	0.038
3.0	6	1.0	2	0.056
4.0	8	0.0	2	0.095

TABLE 4: Rate of Protein Leakage by *Garcinia kola* Seed Crude Extract on the Clinical Isolates

Clinical Isolates	Time Intervals/Absorbance (mm)			
	30minutes	60minutes	90minutes	120minutes
<i>Staphylococcus aureus</i>	0.115	0.336	0.075	0.002
<i>Escherichia coli</i>	0.088	0.365	0.048	0.052
<i>Streptococcus pyogenes</i>	0.082	0.285	0.373	0.035
<i>Salmonella typhi</i>	0.138	0.236	0.082	0.036

TABLE 5: Rate of Protein Leakage by *Garcinia kola* Leaf Crude Extract on the Clinical Isolates

Clinical Isolates	Time Intervals/Absorbance (mm)			
	30minutes	60minutes	90minutes	120minutes
<i>Staphylococcus aureus</i>	0.005	0.262	0.032	0.002
<i>Escherichia coli</i>	0.005	0.212	0.005	0.678
<i>Streptococcus pyogenes</i>	0.032	0.282	0.033	0.032
<i>Salmonella typhi</i>	0.084	0.282	0.015	0.682

TABLE 6: Rate of Potassium (K⁺) ion Leakage by *Garcinia kola* Seed Crude Extract on the Clinical Isolates

Clinical Isolates	Time Intervals/Absorbance (mm)			
	10minutes	20minutes	40minutes	60minutes
<i>Staphylococcus aureus</i>	0.5	6.2	36.2	109.5
<i>Escherichia coli</i>	0.1	6.9	25.7	107.1
<i>Streptococcus pyogenes</i>	3.2	4.8	17.5	110.4
<i>Salmonella typhi</i>	1.9	8.0	34.2	108.0

TABLE 7: Rate of Potassium (K⁺) ion Leakage by *Garcinia kola* Leaf Crude Extract on the Clinical Isolates

Clinical Isolates	Time Intervals/Absorbance (mm)			
	10minutes	20minutes	40minutes	60minutes
<i>Staphylococcus aureus</i>	1.60	2.80	96.20	62.90
<i>Escherichia coli</i>	85.20	75.10	65.01	67.80
<i>Streptococcus pyogenes</i>	12.45	2.82	93.30	32.20
<i>Salmonella typhi</i>	84.50	82.80	65.20	68.20

TABLE 8: Phytochemical Component of *Garcinia kola* Seed and Leaf Extracts

Bioactive Component	Seed Extract				Leaf Extract			
	AA	D	HC	A	AA	D	HC	A
Alkaloids	+	+	-	-	+	+	-	-
Saponins	+	-	-	-	+	-	-	-
Tannins	+	-	-	-	+	-	-	-
Anthraquiones	+	-	-	-	+	-	-	-
Steroids	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	+	-	-	-
Phlobatannins	+	-	-	-	+	-	-	-
Cardiac glycosides:								
Legal Test	+	+	-	-	+	-	-	-
Lieberman Test	+	+	+	+	-	+	+	-
Salkowsla Test	-	-	-	-	-	-	-	-

KEY:

(+) - Present

(-) - Absent

Extracts:

AA – Acetic acid

D – Diethylether

HC – 1% Hydrochloric acid

A – Acetone

Table 9: Zone of inhibition by some selected commercial antibiotics on *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi* and *Escherichia coli*

Antibiotics	Diameter of Zone of Inhibition (mm)			
	<i>S. aureus</i>	<i>S. pyogenes</i>	<i>S. typhi</i>	<i>E. coli</i>
Chloramphenicol (20mg)	-	16	-	11
Augmentin (30mg)	-	-	-	-
Amoxicillin (25mg)	-	-	-	-
Erythromycin (30mg)	-	20	-	12
Ceftilazone (30mg)	-	-	-	-
Nitrofurantoin (200µg)	19	-	-	-
Penfloxacin (5mg)	-	-	14	-
Gentamicin (10mg)	-	-	16	12
Tetracycline (30µg)	-	10	-	14
Cotrimoxazole (25mg)	-	12	-	15

DISCUSSION

The outcome of this study has shown that the seed and leaf extract of *Garcinia kola* possess antibacterial activity. The antibacterial activity against the test organisms varied as the acetic acid extract from the *Garcinia kola* had the highest inhibitory effect on the selected micro organisms. This antibacterial activity against the test organisms is due to the presence of bioactive components present in the extract such as tannin, saponins as shown in this study. This work therefore agrees with Ebanu et al., 1999: "antimicrobial activity in plants results from the bioactive component present in the plants such as alkaloids, saponins, tannins, anthraquinones, steroids, flavonoids etc".

The acetic acid seed extract produced the widest zone of inhibition for gram positive organisms compared to the gram negative organisms. The leaf extract also showed the widest zone of inhibition for gram negative bacteria. This result again showed that the acetic acid extract of *Garcinia kola* had varying activity on gram positive and gram-negative organisms.

The variation in activity of the extract of bitter kola showed that bioactive components had varied degree of solubility in the various solvents used for extraction. It is vital to note that extracts (1% hydrochloric acid and acetone) that produced no zone of inhibition, had no inhibitory component of *Garcinia kola* present in them. For

Diethylether extract that produced small zone of inhibition, had only one bioactive component (alkaloids) present. Acetic acid extract which produced the widest zone of inhibition had bioactive components (tannins, saponins, alkaloids, anthraquinones, phlobatannins and cardiac glycosides) present.

Table 1,2, showed that acetic acid extraction is more effective than other extraction. When an inhibitory zone of less than 10.0mm in diameter is produced by a test organism, the organism is regarded as resistant to the extract. The maximum dilution at which the organism was sensitive to the extract is recorded as the minimum inhibitory concentration (MIC).

The anti bacterial activity of the acetic acid extract of *Garcinia kola* was determined from the result of the diameter of the zones of inhibition, the level of turbidity for each test organisms and the presence of bioactive components. The test organisms *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Salmonella typhi* were all susceptible to susceptibility and turbidity test. These test organisms are clinical isolates which are pathogenic.

Sofowora, 1996 tested the aqueous and alcoholic extracts of *Garcinia kola* on various Gram positive (*Staphylococcus aureus* and beta haemolytic *Streptococci*) and Gram negative bacteria (*Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Neisseria*

gonorrhoeae) and they showed a strong activity against them. Thus, this work therefore agrees with Sofowora,1996.

This work also agrees with Irrine,1981: "dried seed is used for the treatment of dysentery and diarrhoea, while the leaves are used for the treatment of fever". Therefore, the inhibition of the test organisms by the acetic acid extract of *Garcinia kola* agrees with the claim by traditional practitioners and other investigators that the seed and leaf of *Garcinia kola* have antimicrobial activity and could be administered as a form of therapy for the treatment of related diseases of the test organisms since they were all susceptible to all the antimicrobial assay tests.

Adeleke et al 2007, Indabaw, 2011, Ogunjobi, 2011 [14,15,16] assayed the methanolic and ethanolic seed extract respectively of *Garcinia kola* on some selected pathogenic micro organisms including fungi. Their results showed that the seed extracts had antimicrobial activity on the pathogenic micro organisms. Hence, the antibacterial activity of *Garcinia kola*, as shown in this work agrees Adeleke et al 2007, Indabaw, 2011 and Ogunjobi, 2011[14,15,16].

The acetic acid extract activity compared with commercial antibiotics showed a higher inhibitory effect. This is a pointer to the fact that *Garcinia kola* could serve as better therapeutic options than some commercial antibiotics.

CONCLUSION

Garcinia kola has a very promising use as an anti-bacterial agent and for the treatment of infections caused by the test organisms used- *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi* and *Escherichia coli*. Moreover, for the usage of this medicinal plant (*Garcinia kola*) to be fully maximized, it is expected that all parts of the plant should be properly assayed in the laboratory and further research is needed to optimize the effective use of this agent in clinical practice.

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