



Assessment of Antimicrobial Effect of Alcohol and Aqueous Extracts of *Garcinia kola* on *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pneumonia*

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ABSTRACT: As a result of the development of resistance of microorganisms to older antimicrobial agents there is need for a search for new agents, which are effective for the treatment of infections. The crude aqueous and alcoholic extracts of *Garcinia kola* fruits were assayed against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pneumoniae*. The results revealed that the plant extracts possess inhibitory effect against the microorganisms tested. The minimum inhibitory concentration of the plant extracts ranged between 20mg/ml and 45mg/ml. There was a change in the antibacterial activity of the test extracts on variation of temperature. The results obtained may suggest that the plant extract is thermal stable and could serve as a source of industrial drugs useful in chemotherapy of some microbial infections.

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Nature has endowed mankind with a rich store house of natural antimicrobial agents – plants. Plants are vital parts of man’s existence. Dependence on plant for existence has been of paramount importance since the human race began. A medicinal plant is any plant which in one or more of its organs contain substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (Sofowora, 1982). The medicinal use of plants and their products by man came about through observation of definite effect on man and animals. The early man observed that feeding on certain plants caused disturbing or even fatal reactions and such plants are recognized as poisons and employed as such. Thus, from earliest times, tribal priests and medicine men (witch doctors) used various plants, minerals and animal organs usually in association with strange rituals and incantation to drive out the evil spirit which they believe to be the cause of the diseases (Hill, 1952; Kohhar, 1981). Plant parts commonly used include fruits, stem, leaves, seeds and roots. They can be produced in powder mixture, which may be either raw or boiled (concoction or decoction), soup, ointment, liniment or incision. Medicinal plants are used in treatment of diseases either alone or in combination with other plant parts. They are used as anti-infective agents, anti-malarial, laxatives, cardiovascular and nervous remedies, proteolytic ferments, and steroid

sources, sweeter and anti-tumour drugs (Gbile and Adesina, 1986 and Owonubi, 1988). *Garcinia kola* is locally called “Orogbo” in Yoruba, “Odu” in Igbo and “Mijingoro” in Hausa. It is a medium sized tree easily recognized by its fine hairy flower and large fruits, the size and colour of an orange. *Garcinia kola* is one of the antimicrobial agents which have its main effect on bacteria and yeasts (El-said *et al* 1977; Sofowora, 1982; Kolawole, 1982). As a result of ineffectiveness of older antimicrobial agents and drug abuse, there is need for further search for new agents, which are effective for the treatment of infections. The aim of the present study is therefore to investigate the antimicrobial effect of the extracts of *Garcinia kola* on representative organisms *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pneumonia*

MATERIALS AND METHODS

Fruits of *Garcinia kola* used in this study were obtained from Bida, Niger State. Identification was carried out at the Herbarium unit of the Department of Biological Sciences, University of Ilorin, Nigeria. The representative microorganisms used were *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pneumoniae*. Pure isolates of the organisms were obtained from microbiology laboratory, Department of Biological

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Sciences, University of Ilorin. Biochemical and Gram stain tests were used to confirm the identity of the organisms.

Standardisation of bacteria: McFarland standard was used as a reference to adjust the turbidity of bacteria suspension in the range of 1×10^8 bacteria cells/ml and was maintained throughout this study.

Preparation of Plant Extracts: Ten grammes of the powdered dry fruits of *G. kola* were weighed separately into 100ml of 70% ethanol, 70% methanol or distilled water in different screw cap bottles. These were stirred intermittently over a 72 hour period for effective extraction, after which the extracts were filtered through a what-man filter paper number 1, into different screw cap bottles (Akinyanju *et al.*, 1986). Each extract was tested for growth/contamination by plating them on nutrient agar and incubated at 37°C for 24 hours.

Phytochemical analysis: The methods described by Banso and Adeyemo (2006) were used to test for the presence of saponins, tannins, alkaloids, flavonoids and glycosides in the test samples.

Determination of tannins: Ethanolic extract of the plant sample (0.5g) was separately stirred with 10ml of distilled water and then filtered. To the filtrate was added two drops of 5% iron (III) Chloride (FeCl_3) reagent. Blue-black or blue-green colouration or precipitate was taken as an indication of the presence of tannins.

Determination of alkaloids: Extract of the plant sample (0.5g) was separately stirred with 1% hydrochloric acid (HCl) on a steam bath. The solution obtained was filtered and 1ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloid in the extracts.

Determination of saponins: The plant extract (0.5g) was stirred in a test tube, foaming which persisted on working was taken as an evidence for the presence of saponins.

Determination of flavonoids: To ethanolic extract of *Vernonia amygdalina* leaf was added a small piece of magnesium ribbon, this was followed by dropwise addition of concentrated hydrochloric acid. Colours ranging from orange to red indicated flavonols, crimson to magenta indicated flavonones.

Determination of glycosides: Coarsely powdered plant material (1g) was introduced into two different beakers. To one of the beakers was added sulphuric acid (5ml) while water (5ml) was added to the other beaker. The two beakers were heated for three minutes and the content filtered into labelled test tubes. The filtrate was made alkaline with sodium hydroxide (0.5ml) and allowed to stand for three minutes. The presence of reddish brown precipitate in the filtrate was taken as positive for glycosides.

Determination of steroids: A 10ml chloroform extract of the test plant fruit was evaporated to a dry mass and the mass dissolved in 0.5ml chloroform. Acetic anhydride (0.5ml) and 2ml concentrated sulphuric acid were added. A blue or green colour or a mixture of these two shades was regarded as positive for the presence of steroidal compounds.

Determination of terpenoids: The presence of terpenoids was determined as described for steroids except that red, pink or violet colour indicated the presence of terpenoids.

Antimicrobial Test: The antimicrobial test was performed using the agar diffusion method of Banso and Adeyemo (2007a). The test organisms were inoculated on nutrient agar plates and spread uniformly using a sterile glass spreader. Wells of 5mm diameter were made on the nutrient agar using a sterile cork borer the cut agar disks were carefully removed by the use of forceps sterilised by flaming. To each well was introduced various concentrations (5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml, 25mg/ml and 30mg/ml) of the extracts. Negative control experiments comprising the test organisms without the plant extracts were set up; positive control experiments were also prepared with ciprofloxacin (50mg/ml). The plates were allowed to stand for 1h at room temperature for diffusion of the substances to proceed before the growth of the organisms commenced. The plates were incubated at 37°C for 24h. The zones of inhibitions were then recorded.

Determination of Minimum Inhibitory Concentration (MIC) of the Extracts: Different concentrations of the plant extracts were introduced into different test tubes; each tube was inoculated with an overnight culture of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* or *Klebsiella pneumoniae* diluted to give a final concentration 1 million cells per ml. The tubes containing the organisms were incubated at 37°C for 24 hours. The least concentration of the plant extracts that did not permit any visible growth of the inoculated test organism on broth culture was taken as the MIC in

each case. Control experiments with no plant extracts were performed.

Effect of Temperature on Antimicrobial Activity of the Plant Extract: The extracts were heated to 30°C, 60°C, 80°C or 100°C for 30 minutes using a water bath. The antimicrobial activity of the extracts was determined using the agar diffusion method already described.

Statistical Analysis: The data represents mean of three replicates ± standard deviation (SD). The result were subjected to analysis of variance and mean comparisons were performed by Turkey’s multiple range tests using SPSS version 20.0 (statistical package for social science, Inc., Chicago IL, United states). Differences between means were considered significant at P < 0.05.

RESULTS AND DISCUSSION

Phytochemical test: Tannins, alkaloids, flavonoids, glycosides, steroids and terpenoids were detected in the fruit extract of *Garcinia kola* while saponins and flavonoids were absent (Table 1).

TABLE 1: Phytochemical analysis of extract

Active principle	<i>G.kola</i> extract
Tannins	+
Alkaloids	+
Saponins	-
Flavonoids	-
Glycosides	+
Steroids	+
Terpenoids	+

+ = Present; - = Absent

Antimicrobial Activity of Extracts: The antimicrobial test of the fruit extracts of *G. kola* showed that the plant exhibited activities against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pneumoniae* (Table 2). It was observed that an increase

in the concentration of extracts brought more activity as shown in the diameter of zone of inhibition (Table 2). This agrees with the report from Boakye-Yuadom (1979), Kurosaki and Nishi (1983) that higher concentration of antimicrobial substance showed appreciable growth inhibitions. The fact that organisms may need higher concentrations of the extracts to inhibit or kill them may be due to their cell wall component. El-said *et al*; (1977) and Kolawole (1982) showed that *G. kola* has antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pneumonia*

Minimum Inhibitory Concentration (MIC) of the Extracts: Table 3 shows the result of the minimum inhibitory concentration of the extracts. The results indicate that the MIC of aqueous extracts of *G. kola* fruits against *S.aureus*, *B. cereus*, *E. coli* and *K. pneumoniae* were 30mg/l, 25mg/ml, 40mg/ml and 50mg/ml respectively. However, figures of 20mg/ml, 25mg/ml, 30mg/ml and 25mg/ml were obtained for methanol extract of the plant material. MIC values of 25mg/ml, 25mg/ml, 35mg/ml and 45mg/ml were recorded against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pneumoniae* respectively.

The effect of the fruit extracts of *G. kola* on the MIC for the test micro-organisms correlate with the report that microorganisms varied widely in the degree of their susceptibility (El-Faraly *et al.*, 1983). Antimicrobial agents with low activity against an organism have a high MIC. The MIC of methanol extract was relatively lower than those for aqueous and ethanol extracts (Table 3). This suggests that methanol is better than ethanol as extracting solvent for the plant part used in this study.

Table 2: Antimicrobial activity of methanol extract of *G. kola*

Concentration (mg/ml)	Mean diameter of zone of inhibition(mg/ml)			
	SA	BC	EC	KP
5	0.00 ^e	0.00 ^e	0.00 ^d	0.00 ^b
10	9.5±0.2 ^a	9.0±0.1 ^d	0.00 ^d	0.00 ^b
15	10.0±0.1 ^a	11.0±0.1 ^a	12.5±0.1 ^a	13.5±0.1 ^a
20	10.0±0.2 ^a	13.5±0.2 ^b	15.0±0.1 ^b	13.5±0.2 ^a
25	14.5±0.1 ^b	16.0±0.1 ^c	17.5±0.2 ^c	16.5±0.1 ^c
30	17.0±0.2 ^c	17.0±0.2 ^c	18.0±0.1 ^c	18.5±0.2 ^c

SA = *Staphylococcus aureus*, BC= *Bacillus cereus*, EC = *Escherichia coli*, KP = *Klebsiella pneumoniae*; Means on the same column with different superscript are significantly different P < 0.05

Table 3: Minimum inhibitory concentration of methanol fruit extract of *Garcinia kola*

Organisms	Minimum inhibitory concentration (mg/ml)		
	Aqueous extract	Methanol extract	Ethanol extract
<i>S. aureus</i>	30	20	25
<i>B. cereus</i>	25	25	25
<i>E. coli</i>	40	30	35
<i>K. pneumoniae</i>	45	35	40

Effect of Temperature on Antimicrobial Activity of the Plant Extract: Tables 4 show the results of the effect of temperature on the antimicrobial activity of the extracts. There was a change in the antimicrobial activity of the test extracts on variation of temperature of the extracts. The MIC of aqueous extract of *G. kola* increased from 20mg/ml to 25mg/ml after heating from 30°C to 100°C and tested against *B. cereus*. A value of 35mg/ml was recorded against *K. pneumoniae*

when ethanol extract of *G. kola* was heated to 80°C and tested against the bacteria. The effect of temperature on the activity of extracts of *G. kola* against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pneumoniae* (Table 4) indicated that the extracts were more effective at lower temperatures due to the lower values of MIC against the test microorganism. This finding suggests that the antimicrobial substances in the extracts are heat stable.

Table 4: Effect of temperature on the antimicrobial activity of extract

Temp (°C)	Minimum inhibitory concentration (mg/ml)											
	Aqueous extract				Methanol extract				Ethanol extract			
	SA	BC	EC	KP	SA	BC	EC	KP	SA	BC	EC	KP
30	20	20	25	30	20	20	25	20	20	20	20	20
60	25	20	30	35	20	20	25	35	20	20	35	30
80	25	25	35	40	20	20	30	35	25	20	35	35
100	30	25	40	45	20	25	30	35	25	25	35	40

SA = *S. aureus*, BC = *Bacillus cereus*, EC = *Escherichia coli*, KP = *Klebsiella pneumoniae*

Conclusion: Aqueous and alcoholic extracts of *G. kola* fruit extract exhibit antimicrobial property against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pneumoniae*. The antimicrobial substance contained in the extracts exhibited *in-vitro* antimicrobial activity against Gram positive and Gram negative bacteria. These organisms have been implicated in a wide range of infections; it therefore implies that constituents of the fruit extracts of the plant could be useful in chemotherapy bacterial infections.

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