

**Antibacterial activity of Methanolic extract of *Garcinia kola* (Heckel) Seeds and Standard antibiotics.**

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**Running Title: *Garcinia kola* seeds extract and standard antibiotics against bacteria.**

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**ABSTRACT-The methanolic extract of *Garcinia kola* (Heckel) seeds and eight standard antibiotics were tested in-vitro for comparative activity against 10 isolates of each of six bacterial species: *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus pyogenes*, *streptococcus pneumoniae* and *Pseudomonas aeruginosa*, all from throat infections.**

**The methanolic extract exerted activity against all the bacteria tested almost in similar manner as gentamicin. Of the remaining seven standard antibiotics, chloramphenicol, erythromycin, and tetracycline showed activity against one organism or the other. Remarkably, augmentin<sup>R</sup>, cloxacillin and cotrimoxazole had no activity against any of the bacterial isolates. Amoxicillin was able to affect only 2 of the 8 isolates of *Esch. coli*. This has lent credence to the ethnopharmaceutical claims of the curative affect of raw-chewed *Garcinia kola* seeds on throat infections as well as highlighting the bacterial resistance to standard antibiotics, particularly, the  $\beta$ -lactams.**

## INTRODUCTION

*Garcinia kola* (Heckel) is a plant in the family Guttiferae and tropical in its distribution<sup>(1)</sup>. It has various local names in Nigeria but is commonly referred to as bitter/false/male kola. Both the pulp and seeds of the plant are edible<sup>(2)</sup>; the seeds are chewed raw and the roots serve as chewing stick locally. The seed of *Garcinia kola* has been associated with folklore claims on its curative effects. The powdered seeds as well as in the treatment of cough, threatened abortion, diabetes, palpitation, intestinal pains, jaundice, whooping cough, anaemia and angina as well as in the treatment of liver disorders, and as an antidote against poison<sup>(4)</sup>. When chopped up and steeped in water, beer or palm wine, the seed of *Garcinia kola* has a cleansing effect on stomach<sup>(5)</sup> *Garcinia kola* seed extracts have been reported to be active on both Gram-positive and Gram-negative bacteria,

and fungi<sup>(6,7)</sup> attributed principally to such phytochemicals as benzophenone, garcinol and xanthochymol<sup>(8,9,10)</sup>. Specific targeting of this antimicrobial activity against bacterial causative agents of throat infections and a comparative study of such activity with that of standard antibiotics are lacking in the literature. These have formed the basis for this study.

## MATERIALS AND METHODS

### BACTERIA

Sixty bacterial isolates consisting of 10 for every one of 6 bacterial spp were collected from different clinical sources of respiratory tract infections. Some conventional biochemical tests<sup>(11)</sup> were carried out to confirm the identities of the bacteria.

### SOXHLET EXTRACTION OF *GARCINIA KOLA* SEEDS IN ORGANIC SOLVENTS

Sun dried *G. kola* seeds weighing 200g were turned into powdered form on a local hand grater

and blender successively. Extraction with a soxhlet apparatus using 20g of the powder in 200ml of each of methanol, chloroform and petroleum followed by filtration and then, storage at 4°C in a refrigerator until when needed.

#### **CONCENTRATION OF THE METHANOLIC EXTRACT**

The hot filtrate of methanolic extract was concentrated on an

**SUSCEPTIBILITY STUDIES**

The methanolic, chloroform and petroleum ether crude extracts of *Garcinia kola* seed powder, the methanolic extract concentrate and 8 different standard antibiotics (as disks) were used in separate experiments against the bacterial isolates. The preliminary results obtained on the more encouraging antimicrobial activity of the methanolic crude extract than any of the other two necessitated the preferential use of the methanolic extract in its concentrated form. The agar-cup diffusion method

ether, in turns. An exhaustive extraction in each case lasting for about 6 hr was electrothermal water bath to a constant weight of 3.96g. The concentrate was then preserved in a refrigerator at 4°C for further use.

of susceptibility testing was employed on the crude extracts and the concentrate, while antibiotic disk diffusion method was used on the standard antibiotics against the bacteria as previously described<sup>(12)</sup>. The minimum inhibitory concentrations (MIC) of the methanolic extract concentrate against the bacterial isolates were also determined, using double-fold serial dilutions of the concentrate.

#### **RESULTS**

The methanolic extract of *Garcinia kola* seeds had the most

pronounced effect on all the bacteria tested particularly *Esch. coli* and *Staph. aureus*, relative to the effects of the chloroform and petroleum ether extracts. None of the solvents had antibacterial activity on its own. The Duncan's Multiple Range test employed in statistical analysis established a significant difference (at  $P < 0.05$ ) between the methanolic extract and either of the chloroform and petroleum ether extracts. Zone of bacterial growth inhibition (mm) was the parameter used in assessing the antibacterial activity. Among the 8 standard antibiotics used (in disk form), only gentamicin compared

favourably with the methanolic extract of *Garcinia kola* seeds (Table 1). Augmentin<sup>R</sup>, amoxycillin, cloxacillin, erythromycin and co-trimoxazole showed no activity against not less than 8 isolates among each of the bacterial spp. Tetracycline and co-trimoxazole showed no activity against all the isolates of *Strep pyogenes*, *Strep. pneumoniae* and *Pseud aeruginosa*. Chloramphenicol could not affect any one of the isolates of *Pseud aeruginosa* but varied in its activity against other bacteria.

The methanolic extract concentrate gave varying MICs among the microorganisms tested (Table 2).

Table 1

Sample results of susceptibility testing on 10 isolates of each of 3 bacterial spp.

Isolate:	STANDARD ANTIBIOTICS								MET. EXTRACTS		
	GEN	AUG	AMX	ERY	TET	CXC	COT	CHL	O		
PA											
1084	30*	**	-	-	-	-	-	-	13.0	-	
1380	33	-	-	-	-	-	-	-	13.5	-	
2366	28	-	-	-	-	-	-	-	13.0	-	
2819	33	-	-	-	-	-	-	-	11.5	-	
0714	32	-	-	-	-	-	-	-	12.0	-	
3011	29	-	-	-	-	-	-	-	13.5	-	
3681	21	-	-	-	-	-	-	-	13.0	-	
3800	28	-	-	-	-	-	-	-	12.0	-	
4010	33	-	-	-	-	-	-	-	12.5	-	
4499	29	-	-	-	-	-	-	-	13.0	-	
SA											
1122	25	-	-	-	21	-	-	-	13.5	-	
1633	19	-	-	-	10	-	-	-	13.0	-	
2464	27	-	-	-	18	-	-	-	14.5	-	
2994	20	-	-	-	12	-	-	-	16.5	-	
0899	23	-	-	-	11	-	-	-	16.5	-	
3002	25	-	-	-	28	-	-	-	17.0	-	
3533	28	-	-	-	32	-	-	-	16.0	-	
3801	21	-	-	-	19	-	-	-	14.0	-	
4113	22	-	-	-	19	-	-	-	16.0	-	
4430	26	-	-	-	-	-	-	25	17.0	-	
SN											
1164	20	-	-	-	-	-	-	-	8.5	-	

1463	22	-	-	-	-	-	-	-	11.5	-	
2114	20	-	-	-	-	-	-	-	9.5	-	
2845	24	-	-	-	-	-	-	-	10.5	-	
0874	19	-	-	-	-	-	-	-	9.5	-	
3321	22	-	-	-	-	-	-	-	11.0	-	
3644	23	-	-	-	-	-	-	21	10.0	-	
3778	20	-	-	-	-	-	-	26	9.0	-	
4220	10	-	-	-	-	-	-	-	10.5	-	
4400	23	-	-	-	-	-	-	26	10	-	

NB:

- \* = Zone of growth inhibition (mm) (sensitive)  
 \*\* = No growth (No activity)  
 PA = Pseudomonas aeruginosa  
 SA = Staph. aureus  
 SN = Strep. Pneumoniae  
 GE = Gentamicin (10µg); AU = Augmentin<sup>R</sup> (30µg)  
 AM = Amoxycillin (25µg); ER = Erythromycin (5µg)  
 TE = Tetracycline (10µg); CX = Cloxacillin (5µg)  
 CO = Cotrimoxazole (25µg); CH = Chloramphenicol (30µg)  
 ME = EXTRACT = Methanol extract  
 C = Control (Methanol)

**Table 2**

**Sample result of MIC determination for Methanolic extract concentrate**

**Isolate: MITC (mg/ml)                      Isolate MIC (mg/ml)**

**PA**

1084	75	2819	49.50
1388	49.50	714	49.50

2366	49.50		
SA			
1122	24.75	2994	49.50
2464	24.75	1633	24.75
0899	49.50		

SN			
1164	99.00	2845	
99.50			
12463	49.50	0874	99.50
2114	99.00		

NB:

PA = *Pseudomonas aeruginosa*

SA = *Staph. aureus*

SN = *Strep. pneumoniae*

#### DISCUSSION

The pronounced antibacterial effect of the methanolic extract of *Garcinia kola* seeds on all the bacterial isolates tested, including Gram - positive and Gram negative bacteria, has significantly lent credence to the ethnopharmaceutical claims of the curative effect of the raw chewed

*Garcinia kola* seeds on respiratory tract infections<sub>(3)</sub>. The observation in this study that only gentamicin of all the eight standard antibiotics tested, could compare favorably with the methanolic extract emphasizes the earlier observed<sub>(13)</sub> worrisome dimension in bacterial antibiotic resistance, particularly to the  $\beta$ -

lactams. Most importantly, *Staph aureus*, *Strep. pyogenes*, *Strep. pneumoniae* and *Pseud. aeruginosa* have deservedly, due to their clinical importance, enjoyed attraction for their resistance to antibiotics. This has been substantiated in this study with respect to virtually all the  $\beta$ -lactams (augmentin<sup>R</sup>, amoxicillin and cloxacillin) tested. It is interesting to note that the methanolic extract exhibited pronounced activity against *Esch. coli*, the hospital isolates of which were found to be resistant to amoxicillin – clavulanate<sub>(14)</sub> the same as Augmentin<sup>R</sup> There is need to exploit fully the therapeutic value of *Garcinia kola* seeds through definitive isolation of the active principle and its formulation into suitable dosage forms for the treatment of respiratory tract infections.

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