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Antimicrobial Activity of the Crude Extracts and Isolated Fractions of Garcinia Kola Heckel Stem Bark

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Abstract

The stem bark of Garcinia kola, commonly used for various ailments in traditional medicine in Nigeria was examined for antimicrobial activity. Crude chloroform and methanolic extracts and fractions isolated from the chloroform extract using column chromatography were screened for in-vitro antibacterial and antifungal activities. As tested using agar cup diffusion method on *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 9001), *Pseudomonas aeruginosa* (NCTC 6750), *Bacillus cereus* (Lab. Stock), *Candida albicans* (NCTC 7534) and Aspergillus niger (Lab. Stock), the crude extracts showed good activity at 10 and 20mg/ml respectively. The 14 fractions isolated from the column chromatography also showed varying but significant degree of activity on most of the tested organisms at 100µg/ml. The study has shown that *G. Kola* bark has antimicrobial activities that justify its uses in traditional medicine in Nigeria as anti-infective agent. Also, the fact that most fractions showed antimicrobial activity is an indication that *G. Kola* bark has many antimicrobial constituents which are different but closely related.

Introduction

Plants have been utilized since time immemorial for curing diseases. Even today nearly 70% of the world's population is dependent on plants for handling their health related problems (Fabricant and Farnsworth, 2001).

Garcinia kola Heckel (Guttiferae) seeds and barks have been known for its medicinal attributes in Nigeria and elsewhere as anti-infective, anti-hepatotoxic, anti-diabetic, anti-inflammatory and so on (Dalziel, 1948; Igboko and Iwu, 1986; Burkhill, 1994; Iwu, 1993; Iwu, 2002). Previous research on *G. cola* has been on the seeds leading to isolation of complex mixture of biflavonoids, prenylated benzophenones, and xanthones like kolanone, garcinol and xanthochymol (Hussain *et al*, 1982; Iwu *et al*, 1990; Iwu, 2002) most of which showed antibacterial property.

Madubunyi (1995), showed that the MIC of GB1 (a garcinial biflavone) on *Staphylococcus aureus* and *E. coli* were 3.1 x 10-7 and 3.0 x 10-3µg/ml respectively. While Akoachere, *et al* (2002) reported the MIC of the seed extract on respiratory tract pathogens to be from 8.0 x 10^{-5}µg/ml to 1.8µg/ml.

However, not much work has been reported on the barks which are also regularly employed in traditional treatment of infectious diseases (and other ailments). We hereby report the results of screening crude extracts and isolated fractions of G. kola stem bark on common pathogenic bacteria and fungi.

Materials and Methods

Plant Materials

Stem barks were peeled from a big *Garcinia kola* tree located at Iragbiji, Osun State, Nigeria during the raining seasons. Using the leaves, fruits and flowering tops, the plant was authenticated in the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, Nigeria where herbarium voucher specimens were deposited.

The plant samples were prepared as described by Williamson *et al*, 1996. The dried, pulverized bark (350g) was extracted with successive quantities of chloroform and methanol using soxhlet apparatus. The extracts were concentrated in vacuo, air dried to constant weight and were stored at 4°C for use.

Micro-organisms and Media

Staphylococcus aureus (NCTC, 6571), *Escherichia coli* (NCTC, 9001), *Pseudomonas aerughosa* (NCTC, 6570) and *Candida albicans* (NCTC, 7534) were obtained from University of Strathclyde, Glasgow while Bacillus cereus and *Aspergillus niger* were from the laboratory stock of the Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria.

All the media: Nutrient Agar (No. 2) and Nutrient Anith (pH. 7.4) used for bacteria; and Sabouraud Dextrose Agar (pH 5.4) and Triptone Soy broth used for fungi were from Oxoid Laboratories, England.

Preliminary phytochemical screening

The powdered stem bark was tested for the presence of the following secondary metabolites; alkaloids, anthraquinone glycosides, tannins, saponins, cardenolides and flavonoids using standard procedures (Harbone, 1984; Sofowora, 1982).

Column and TLC chromatography

Chloroform extracts of G. kola was first analyzed on a precoated TLC plate, silica gel G F254(Sandoz, Basel) using a solvent system containing Hexane: Chloroform: Ethyl acetate: methanol (4:3:2:1) as mobile phase to effect a good separation yielding twelve different spots whose characteristics are shown in Table 2. The crude extracts were dissolved in CHCl3-MeOH (1:1) solvent for spotting following standard procedures (Touchstone, 1992). Column chromatography of the chloroform extract on silical gel G 60 (70-230 mesh, ASTM) eluting with hexane – chloroform – methanol gradients of increasing polarity afforded a total of 100 fractions of 10mls each. The eluates were monitored on TLC plates and fractions with similar component were bulked together to give a final 14 different fractions which were tested for antimicrobial activity. The characteristics of each fraction labeled A1 – N are shown in Table 3.

Antimicrobial Screening

Using agar-cup diffusion method (Perez, *et al*, 1990), a 0.1ml of a two-fold dilution of overnight broth culture of each bacteria and fungi was seeded into nutrient broth and Sabourauds dextrose agar respectively. A sterile cork borer (diameter 6mm) was used to punch uniform wells on the set and dried agar. Each well was filled with a 0.2ml of the crude extracts (10 and 20mg/ml) and the isolated fractions (100µg/ml) using methanol as solvent. Ampicillin (Beecham, England) and Tioconazole (Pfizer, New York) at 2.5 and 0.5µg/ml respectively were used as control antimicrobial agents. While testing the fractions, 10mg/ml of the crude chloroform extract (X) was also tested along with the fractions for comparison purpose. Bacterial plates were incubated at 37°C for 24hrs while fungal plates were incubated at 25°C for 24 – 72 hrs. All tests were performed in triplicates and the average diameter of the zones of inhibition was used as a measure of antimicrobial activity.

Results and Discussion

The pulverized bark yielded 4.4% to chloroform and 17.8% to methanol. The higher yield of methanol compared with choloroform is an indication that most of the constituents are in polar form e.g phenolics and tannins as shown by the results of the phytochemical screening for secondary metabolites (Table 1). The TLC analysis of the chloroform extract shown in Table 2 revealed the presence of at least 12 different compounds which was a guide to the number of fractions expected from column chromatographic isolation (Table 3). Fractions from column chromatography when monitored by TLC analysis showed that fractions A, B and F were single fractions while others contained some overlaps that required further purification.

Antimicrobial screening of the crude extracts showed that the methanolic extracts (Table 4) was more active than the chloroform extract. It was noted that the extracts showed a broad spectrum of antimicrobial activity that cut across all the organisms which include Gram-positive and Gram-negative bacteria, mold and yeast. Similarly, some of the isolated fractions (A, B and G) also displayed the type of broad spectrum antimicrobial activity recorded for the crude extracts (Table 5). All the tested fractions (Except, F) showed a good activity against *Candida albicans* comparable to that of Tioconazole. Also of special interest is fraction F, a reddish brown compound eluted by chloroform-methanol (90 – 10) with good activity against Gram-positive *Staph. aureus* and *B.cereus* comparable to ampicillin but without activity against Gram-negative *Ps. aeruginosa* and *E. coli* and *Candida albicans*. In contrast, fractions H, M and N showed activity against Gram-negative *Ps. aeruginosa* and *E.coli* and *Candida albicans* but with no activity on the Gram-positive *Staph. aureus* and *B. cereus* tested.

Most of the fractions also showed better antimicrobial activity than the crude extract X as compared while some fractions showed less activity. This may be an indication of synergy, interference and antagonism among the constituents of *G. kola* bark.

Table 1: Phytochemical Screening of *G. kola* Stem Bark

	Alkaloids	Cardiac Glycosides	Flavonoids	Anthraquinone Glycosides	Saponins	Tannins
G. kola	+	-	++	-	++	++

Key: ± trace - absent ++ present.

Table 2: TLC Analysis of Chloroform Extract of *G. kola* Stem Bark

Spots	Rf	Colour (uv) 365nm	Colour (iodine tank)
A	0.89	Blue	Colourless
B	0.80	Yellow	Colourless
C	0.76	Yellow	Red
D	0.58	Colourless	Green
E	0.54	Yellow	Grey
F	0.47	Colourless	Brown
G	0.39	Brown	Yellow
H	0.25	Colourless	Brown
I	0.22	Brown	Grey
J	0.17	Colourless	Grey
K	0.05	Colourless	Grey
L	0.03	Colourless	Brown

Mobile phase = Hexane : Chloroform : Ethyl acetate : Methanol
4 3 2 1

Stationery phase = Silica Gel G. (precoated phase, Based)
Temperature 25^{0}C Visualization : Uv-light 365nm and iodine vapour

Table 3: Column Chromatographic Separation of Crude Chloroform Extract

Eluted fractions	Solvent System Used %	Colour of Fractions (in daylight)	Fractions label
1 – 4	Hexane (100)	Colourless	A
4 – 10	Hex- Chloc. (80 – 20)	Colourless	A
21- 38	Hex- Chloc. (60 – 40)	Colourless	A
39 - 55	Hex- Chloc. (60 – 40)	Yellow	A
56 - 70	Hex- Chlor (20 - 80)	Light Yellow	D
71 – 76	Hex- Chlor. (0 – 100)	Yellow	E
77	Chlor-MeOH (90 – 10)	Reddish Brown	F
78 – 81	Chlor-MeOH (30 – 20)	Yellow	G
82	Chlor-MeOH (80 - 20)	Light Yellow	H
83 – 84	Chlor-MeOH (80 – 20)	Yellow	I
85 – 88	Chlor-MeOH (70 – 30)	Light Yellow	J
89 – 92	Chlor-MeOH (50 – 50)	Colourless	K
93 – 95	Chlor-MeOH (10 – 90)	Colourless	L
95 – 100	Chlor-MeOH (0 – 100)	Cololurless	M

Table 4: Antimicrobial Screening of Crude Extracts of *G. Kola*

Extracts	Conc. Used	Diameter Zone of Inhibition in mm					
		Staph. aureus	*Bacillus cereus*	*Escheri coli*	*Pseudomonas aeriginosa*	*Aspergillus niger*	*Candida albicans*
Methanolic	20mg/ml	20.0	16.0	22.0	11.0	18.5	15.0
Chloroform	20mg/ml	12.0	12.0	15.0	9.0	12.0	9.0
Methanolic	10mg/ml	11.0	10.0	18.0	9.0	NT	NT
Chloroform	10mg/ml	9.0	10.5	10.0	8.0	NT	NT
Ampicilin	2.5mg/ml	22.0	20.5	21.0	-	-	19.0
Tioconazole	0.5mg/ml	NT	NT	NT	NT	19.0	19.0

Key: - = Not Active, NT – Not Tested

Table 5: Antimicrobial Activity of Isolated Fractions of Chloroform Extract

Fractions	Diameter Zone of Inhibition (mm)				
	Staph. Aureus	*Bacillus cereus*	*E. coli*	*Ps. aeniginosa*	*Candida albicans*
A	13.0	11.0	10.0	14.0	16.0
B	8.0	10.0	8.0	10.0	NT
C	12.0	14.0	-	-	NT
D	10.0	12.0	12.0	-	15.0
E	8.0	12.0	-	8.0	14.0
F	16.0	19.0	-	-	-
G	10.0	8.0	10.0	10.0	16.0
H	-	-	8.0	8.0	12.0
I	10.0	10.0	-	8.0	11.0
J	10.0	-	8.0	-	12.0
L	8.0	8.0	-	-	10.0
M	-	-	8.0	10.0	8.0
N	-	-	10.0	10.0	8.0
X	12.0	10.0	12.0	12.0	8.0
Ampicillin	22.0	20.5	21.0	NT	19.0
Tioconazole	NT	NT	NT	NT	20.0

Key: - = Not Active; NT = Not Tested

Conclusion

This study has shown that the stem barks of *Garcinia kola* (and not only the seeds) also possess broad spectrum antimicrobial activity, which justify the use of the bark in traditional medicine as anti-infective agent.

Also, the fact that all the fractions possess antimicrobial activity showed that *G.kola* bark possess as considerable number different compounds that possess antibacterial and or antifungal properties. With further purification of some of the fractions, characterization and identification, the active constituents could be named and compared with already identified bioflavonoids, prenylated benzophenones and xanthones of *Garcinia* species.

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