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Full Length Research Paper

Antimicrobial activity of the ethanol extract and fractions of the seeds of *Garcinia kola* Heckel (Guttiferae)

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The crude ethanol extract, aqueous and chloroform fractions of the seeds of *Garcinia kola* Heckel (Guttiferae) was investigated for antimicrobial activity. Agar well diffusion and minimum inhibitory concentration determinations were the methods employed for the study. Clinical bacterial and fungal isolates were used as indicator organisms while standard antimicrobial agents were included in the study. The crude ethanol extract showed significant inhibitory activity against clinical isolates of both Gram positive and Gram negative organisms. It was active against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus viridans*, *Escherichia coli*, *Pseudomonas aeroginosa* and *Klebsiella pneumonia*. It also showed significant inhibitory activity against fungi like *Penicillium notatum*, *Aspergillus niger* and *Candida albicans*. Both the aqueous and chloroform fractions showed activity against the clinical isolates of *S. aureus*, *E. coli* and *P. aeruginosa*. The MIC values obtained ranged between 2.5 and 7.5 mg/ml for bacteria and fungi isolates. The results showed that the crude ethanol extract has broad spectrum of activity, while the fractions exhibited narrow spectrum of activity, since they were active against *S. aureus*, *E. coli*, and *P. aeruginosa* only. These observations could be the basis for the usefulness of the seeds of *G. kola* in the treatment remedies for microbial infections.

Key words: *Garcinia kola*, ethanol extract, antimicrobial activity.

INTRODUCTION

Garcinia kola Heckel (Guttiferae) commonly known as Bitter Kola is a medium-sized tree mostly about 12 m high and sometimes up to 28 m in height. The bark is thick and brownish, yielding a yellow juice. The leaves are broadly elliptic, acute or shortly acuminate at the apex. The fruits are reddish yellow and about 6 cm in dia-meter with 2 - 4 brown seeds embedded in an orange coloured pulp (Keay et al., 1989).

The plant has a variety of local uses. The dried fruit is used to treat arthritis (Ebana et al., 1991). The pulp is used externally as an antiseptic for cuts and sore throats (Iwu, 1990). The bark of *G. kola* is taken orally for fever, cough, inflammation, respiratory tract disease and as an antihelmintic (Gill, 1986). The dried root soaked in local gin is taken orally for the treatments of coughs, inflammation, liver cirrhosis, tooth decay and gonorrhea (Iwu et al., 1990). Biological activity reported for the plant includes the use of the methanolic extract of the dried leaf as a molluscicide (Okonji and Iwu, 1988). Lutete et al. (1994) reported the antibacterial activity of the tannin fraction of the dried stem bark of *G. kola* against *Escherichia piracoli*, *K. pneumoniae*, *Shigella flexneri*, and *Staphylococcus*. Kolaviron, a *G. kola* extract, has been reported to significantly prevent hepatotoxicity induced by several hepatotoxic agents such as phalloidin, thioacetamide and paracetamol (Akintonwa and Essien, 1990). Kolaviron has also been shown to have *in vitro* antioxidant action (Arouma et al., 1990).

The antibacterial and anti-fungal activity of the water

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extract of the half dried twigs of *G. kola* against *Fusobacterium nucleatum*, *Prevotella intermedia*, *Campylobacter gingivalis* has also been reported (Sote and Wilson, 1999). The present study examined the antimicrobial activity of the ethanol extract and fractions of the seeds of *G. kola*, against a range of bacterial and fungal organisms.

MATERIALS AND METHODS

Plant material

The seeds of *G. kola* were collected in March, 2007 at Benin City, Nigeria. The seeds were identified by Mr. Sunday Nweke of the department of pharmacognosy, faculty of pharmacy, university of Benin. A voucher specimen of the plant has been deposited at the University of Benin herbarium. The seeds were cut into bits and dried in the open for 5 days. The dried seeds were then powdered using an electric mill.

Preparation of the total extract

290 g of *G. kola* seed powder was extracted with 1.2 L of 95% etha-nol over 72 h. The extract was reduced to dryness in a rotary eva-porator to yield 36.20g (12.48%, w/w) of the crude ethanol extract.

Fractionation of the total extract

34 g of the crude ethanol extract was partitioned into chloroform and water (1:1) using a separating funnel. The separated fractions were concentrated to dryness using a rotar vapour. The yield of the chloroform fraction was 2.18 g (6.4% w/w) and that of the aqueous fraction was 3.02 g (8.9% w/w). The crude ethanol extract and the separated fractions were used for the antimicrobial studies.

Test organisms

The organisms used in this study were clinical isolates of *S. aureus*, *B. subtilis*, *S. viridans*, *E. coli*, *P. aeruginosa* and *K. pneumonia*. The fungal isolates include *A. niger*, *Penicillum spp* and *C. albicans*. These were all supplied by the Pharmaceutical Microbiology Department of the University of Benin, Benin City.

Drugs

Amoxicillin, (Smithkline Beecham Pharmaceuticals, UK); Ciprofloxa-cin, (Ranbaxy Pharmaceuticals, India); fluconazole (Drug field Phar-maceuticals, Nigeria).

Microbiological media

Nutrient broth, nutrient agar, Sabouraud dextrose agar, sabouraud dextrose broth (Oxoid, England).

Antimicrobial testing

The agar-well diffusion method was used to determine the antimicrobial activity of the extracts (Irobi et al., 1996; Russell and Fur, 1977). The bacterial isolates were first grown in nutrient broth for 18 h. 0.2 ml of the log phase culture was aseptically used to seed a molten nutrient agar which had been cooled to about 45^{o}C, mixed gently and poured into sterile petri dishes and allowed to set. The extracts were tested at 50 mg/ml concentration. This was delivered into wells (8 mm in diameter) bored unto the surface of the already seeded nutrient agar plates.

Standard antibiotics concentration of ciprofloxacin 25 mg/l, amoxycillin 25 mg/l and fluconazole 5 mg/l were assayed using the agar-well diffusion technique. The fungal isolates were treated in a slightly different way. They were first grown on Sabouraud dextrose broth, before they were assayed using Sabouraud dextrose agar. The bacterial plates were incubated at 37^{o}C for 24 h, while the fungal plates were incubated at 25^{o}C (room temp.) for 72 h. The zones of inhibition were measured in mm diameter and recorded.

Determination of minimum inhibitory concentrations (MICs)

The agar dilution method was also used (Russell and Fur, 1977). The extracts were incorporated into molten nutrient agar at concentrations of 40, 30, 20, 15, 10, 7.5, and 5.0, 2.5 mg/ml aseptically, mixed gently in sterile Petri-dishes, and then allowed to set. The surface of the agar plates were allowed to dry properly before inoculating with appropriate bacterial and fungal culture previously diluted to about 10^{6} cfu/ml. The plates were then incubated at 37^{o}C for up to 72 h after about 30 min of inoculation. The lowest concentration preventing visible growth in each determination was taken as the minimum inhibitory concentration. The mean of three replicate determinations was obtained.

RESULTS AND DISCUSSION

The results for the antimicrobial sensitivity tests (inhibition zone diameters (mm)) for the extracts (at 50 mg), standard antimicrobial doses, ciprofloxacin 25 mg, amoxycillin 25 mg, fluconazole 5 mg against species of Gram negative bacteria, Gram positive bacteria and fungi are given in Table 1. The solvents used for the dissolution of the extract did not show any activity at the volume used. The crude ethanol extract was the most active of the three extracts showing activity against all the isolates. The highest activity was against fungi species, especially, *Penicillum notatum*. The aqueous and chloroform fractions were active against *E. coli*, *P. aeruginosa* and *S. aureus*. The aqueous and chloroform fractions were however not active against fungi.

The results further showed that the ethanol extract exhibited almost similar activity (zone diameters) as amoxycillin against *S. aureus*, *B. subtilis*, *S. viridans and E. coli*. The ethanol extract has obvious antibacterial and antifungal activities at tested concentrations. The activities of ethanol extract, aqueous fraction and chloroform fraction against *S. aureus* were, in descending order viz; crude ethanol extract > aqueous fraction > chloroform fraction. The activity of ethanol extract against Gram-positive organisms was in descending order of *S. aureus>S. viridians>B. subtilis*. Whereas the activity against fungi strains were in the order, *P. notatum>A. niger >C. albicans*. Also, the various extracts showed better activity against the Gram negative organisms, when compared with the standard amoxicillin, since the latter only showed

Table 1. Antimicrobial activity of *Garcinia kola* extract and standard antimicrobial agents.

Microorganisms	Zone of Inhibition (mm) ± SD					
	CEE (50 mg/ml)	AF (50 mg/ml)	CF (50 mg/ml)	CP (25 mg/ml)	AMX (25 mg/ml)	F (5 mg/ml)
Staphylococcus aureus	20±4.4	20±2.4	16±2.8	31±4.2	21±1.0	N.D
Bacillus subtilis	15±1.0	-	-	33±4.4	17±2.9	N.D
Streptococcus viridans	15±2.3	-	-	33±4.6	19±3.6	N.D
Escherichia coli	17±4.2	15±4.2	13±2.2	25±1.6	18±2.8	N.D
Pseudomonas aeruginosa	19±4.2	17±2.6	16±2.1	35±2.0	-	N.D
Klebsiella pneumoniae	17±2.1	-	-	27±1.0	-	N.D
Penicillum notatum	26±2.8	-	-	N.D	N.D	32±1.9
Aspergillus niger	22±3.5	-	-	N.D	N.D	28±2.0
Candida albicans	22±2.1	-	-	N.D	N.D	33±1.2

CEE, Crude ethanol extract; AF, aqueous fraction; CF, chloroform fraction; CP, ciprofloxacin
AMX, amoxicillin; F, fluconazole.
- = No Inhibition; ND = Not determined

Table 2. Minimum Inhibitory concentrations (MICs) of the extracts of *Garcinia kola*.

Microorganism	MIC (mg/ml)					
	Crude extract		Water fraction		Chloroform fraction	
	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90
Staphylococcus aureus	2.5	2.5	2.5	2.5	5.0	5.0
Bacillus subtilis	7.5	7.5	-	-	-	-
Streptococcus viridans	2.5	2.5	-	-	-	-
Escherichia coli	5.0	5.0	7.5	7.5	7.5	7.5
Pseudomonas aeruginosa	2.5	2.5	7.5	7.5	7.5	7.5
Klebsiella pneumoniae	5.0	5.0	-	-	-	-
Penicillum notatum	2.5	2.5	-	-	-	-
Aspergillus niger	2.5	2.5	-	-	-	-
Candida albicans	2.5	2.5	-	-	-	-

- = No Inhibition, hence no MIC. Mean of three replicate determinations.

activity against *E. coli* at the dose tested.

The minimum inhibitory concentrations (MICs) of the extracts and fractions are shown in Table 2. This varied between 2.5 and 7.5 mg/ml against all the bacterial and fungi strains used in this study. The MIC of ethanol extract was 25 mg/ml against all the fungal strains. The superior activity of ethanol extract compared with aqueous and chloroform fractions were clearly demonstrated against the tested microorganisms. This was clearly obvious with *P. aeruginosa*, even though there was apparent equipotency of the ethanol extract and aqueous fraction against *S. aureus*. The broad spectrum of activity displayed by the ethanol extract in this study would appear to justify and explain the scientific basis for some of the uses of the other parts of *G. kola* Heckel, such as its use in the treatment of skin infection by some native folks in Benin City.

There is as yet no literature report on the antimicrobial activity neither of the ethanol extract, nor of either the aqueous or chloroform fractions. The earlier antimicrobial reports were on dried root decoction in the treatment of tooth decay and gonorrhea (Iwu et al., 1990), and antibacterial activity of tannin fraction of the dried stem barks (Lutete, et al., 1994). Reports have shown that *Garcinia conrauana* possessed alkaloids biflavonoid, saponins and fibres (Yang et al., 1987). It was also reported to cause a mild improvement in airflow in asthmatic adult males when ingested (Ebomoyi et al., 2004). The antimicrobial active-ties observed in this study coupled with earlier reported physiological activities would find useful purpose in mixed respiratory infections, especially in asthmatics with under-lying bacteria pathogens sensitive to remedies/recipes prepared with *G. kola* ethanol extract for this purpose. Further studies on the isolation and further characteri-zation of the bioactive substances in the crude ethanol extract and concomitant aqueous and chloroform frac-tions would be undertaken.

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