Biocidal activity of partially purified fractions from methanolic extract of *Garcinia kola* (Heckel) seeds on bacterial isolates

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ABSTRACT

The *in vitro* antibacterial activity of crude methanolic extract of the seeds of *Garcinia kola* was investigated. The extracts exhibited antibacterial activities with zones of inhibition ranging from 10 mm to 25 mm. The minimum inhibitory concentration of the diethyl ether fraction was between 0.313 and 5.0 mg/ml, while that of butanol fraction varied from 0.157 to 5.0 mg/ml. The butanol fraction killed about 77% of *Bacillus anthracis* and 79% of *Escherichia coli* cells within 120 min at a concentration of 5.0 mg/ml. Protein leakage from the *B. anthracis* and *E. coli* cells when exposed to the butanol and diethyl ether fractions was observed. We conclude that *Garcinia kola* seed extract has a broad spectrum antibacterial activity, with the butanol and diethyl ether fractions being bactericidal as exemplified by the killing rate and protein leakage regimes, which suggest cell membrane disruption as a mechanism of action of the extract.

Key terms: *Garcinia kola*, antimicrobial activity, MIC, killing rate, protein leakage.

1.0 INTRODUCTION

Medicinal plants have been used as folklore remedies over the years to treat, manage or control man's ailments. They contain large varieties of chemical substances that possess important therapeutic properties used in the treatment of these ailments. Also, the problem of bacterial resistance to commonly used antibiotics has necessitated the search for newer and alternative compounds for the treatment of drug resistant infections and the high cost of conventional drugs, particularly in resource poor communities of the African continent has led to the increased use of plants as an alternative for the treatment of infectious diseases (Sibanda and Okoh 2008a). Several findings on the chemotherapeutic potentials of plants have shown that they can be sources of antimicrobial compounds

of value (Rabe and van Staden, 1997). A typical example of such plants is *Garcinia kola*.

Garcinia kola belongs to the family Guittiferae, and it is commonly referred to as bitter kola. The plant has the popular acronym "wonder plant" amongst the south-western Nigerian people because every part of it has been found to be of medicinal importance (Dalziel, 1937). *G. kola* is used in folklore remedies for the treatment of ailments such as liver disorders, hepatitis, diarrhoea, laryngitis, bronchitis and gonorrhoea (Iwu, 1993; Adesina et al., 1995). The seed is masticatory and is also used to prevent and relieve colic, chest colds, and cough and can be used as well to treat headache (Ayensu, 1978). Iwu (1993) reported the use of this plant for the treatment of jaundice, high fever, purgative and chewing stick. The plant has also been

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found useful in the treatment of stomach ache and gastritis (Ajebesone and Aina, 2004).

Aqueous, ethanolic and petroleum ether extracts of the seeds have been observed to possess antibacterial properties (Ezeifeka et al., 2004), and Kolaviron, (a fraction of the defatted ethanol extract of the seed, containing Garcinia biflavonoid GB1, GB2 and kolaflavanone) has been reported to possess numerous therapeutic potentials (Farombi et al., 2002; Uko et al., 2001), along with such components as mixtures of phenolic compounds, biflavonoids, xanthenes, benzophenones and related triterpenes (Han et al., 2005).

While numerous studies have been done on the antimicrobial potential of this plant, the overwhelming majority of the studies have concentrated on oral and respiratory tract pathogens (Akoachere et al., 2002; Ndukwe et al., 2005), thus underestimating the antimicrobial potential of the plant. As well, reports so far available on the antibacterial potential of the seed show that activity has been demonstrated for the aqueous, ethanolic, acetone and petroleum ether extracts of the seed (Sibanda and Okoh, 2008a,b; Ezeifeka et al., 2004). There are no documented reports in literature on the antimicrobial potential of the methanol extract of the seeds, or fractions thereof. In addition, while previous researchers have used MICs and MBCs as prediction tools for antimicrobial action of plant extracts, there are limitations in the use of such data since it does not consider time-related antimicrobial effects (Kiem and Schentag, 2006), such as killing rate. In this paper, we report the biocidal activity of crude methanolic extracts of the seeds of *Garcinia kola*, and use the killing rate of the extract and protein leakage from cells to predict their bactericidal efficiency.

2.0 MATERIALS AND METHODS

2.0.1 Plant material

Fresh seeds of *G. kola* were used in this work. The seeds were peeled and cut into pieces and later dried in a hot-air oven at 40

°C to constant weight, powdered and kept in an air-tight container for further use.

2.0.2 Preparation of the plant extract

Exactly 850 g of the powdered seeds was soaked in a mixture of methanol and sterile distilled water in a 3:2 ratio for four days and later filtered to obtain the methanolic extract. The mixture was first concentrated *in vacuo* using a rotary evaporator to remove the methanol. The aqueous residue was then lyophilized to obtain a 7.2 g yield of a dark brown crude extract.

2.0.3 Preparation of test microorganisms

The following typed cultures and locally isolated (LIO) organisms obtained from the culture collection of the Microbiology Department, Obafemi Awolowo University, Ile Ife, Nigeria were used for this study:

Gram-positive: *Bacillus subtilis* (NCIB 3610), *Staphylococcus aureus* (NCIB 8588), *Enterococcus faecalis* (NCIB 775), *Micrococcus luteus* (NCIB 196), *Bacillus cereus* (NCIB 6349), *Bacillus stearothermophilus* (NCIB 8222), *Bacillus polymyxa* (LIO), *Bacillus anthracis* (LIO), *Staphylococcus epidermidis* (LIO), *Clostridium sporogenes* (NCIB 532), *Corynebacterium pyogenes* (LIO).

Gram-negative: *Escherichia coli* (NCIB 86), *Pseudomonas aeruginosa* (NCIB 950), *Pseudomonas fluorescens* (NCIB 3756), *Klebsiella pneumoniae* (NCIB 418).

For the experiment, the bacterial isolates were first subcultured in nutrient broth (Oxoid) and incubated at 37 °C for 18 h.

2.0.4 Phytochemical assays

A small portion of the dry extract was subjected to the phytochemical test using the methods of Trease and Evans (1983), and Harbourne (1983) to test for alkaloids, tannins, flavonoids, steroids, saponins, reducing sugars and cardiac glycoside.

2.0.4.1 Test for alkaloids

Exactly 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl in a steam

bath. One millilitre of the filtrate was treated with drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloids.

2.0.4.2 Test for tannins

About 1 g of the extract was dissolved in 20 ml of distilled water and filtered. Two to three drops of 10% of FeCl_3 was added to 2 ml of the filtrate. The production of a blackish-blue or blackish-green colouration was indicative of tannins. To another 2 ml of the filtrate was added 1 ml of bromine water. A precipitate was taken as positive for tannins.

2.0.4.3 Test for flavonoids

About 0.2 g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture, followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange colouration was indicative of the flavonoids.

2.0.4.4 Test for saponins

Freshly prepared 7% blood agar medium was used and wells were made in it. The extract in methanol was applied with distilled water and methanol used separately as negative controls, while commercial saponin (BDH) solution was used as positive control. The plates were incubated at 35°C for 6 h. Complete haemolysis of the blood around the extract was indicative of saponins.

2.0.4.5 Test for steroids

The method of Igwe et al. (2007) was used to test for steroids. About 0.5 g of the extract was dissolved in 3 ml of CHCl_3 and filtered. To the filtrate was added concentrated H_2SO_4 to form a lower layer. A reddish brown colour was taken as positive for steroid ring.

2.0.4.6 Test for cardiac glycoside

About 0.5 g of the extract was dissolved in 2 ml of glacial acetic acid containing 1 drop of 1% FeCl_3 . This was underlaid with conc.

H_2SO_4 . A brown ring obtained at the interface indicated the presence of a deoxy sugar, characteristic of cardiac glycosides. A violet ring may appear below the ring while in the acetic acid layer; a greenish ring may form just above ring and gradually spread throughout this layer.

2.0.4.7 Test for reducing sugars

One millilitre each of Fehling's solutions I and II was added to 2 ml of the aqueous solution of the extract. The mixture was heated in a boiling water bath for about 2-5 minutes. The production of a brick red precipitate indicated the presence of reducing sugars.

2.0.5 Fractionation of crude methanolic extract

The crude extract was successfully partitioned using organic solvents in order of their polarity, that is, n-hexane, chloroform, diethyl ether and butanol. Exactly 90 g of the crude extract was resolved in 100 ml of sterile distilled water in a 500 ml separatory funnel. This was allowed to dissolve properly and later about 100 ml of n-hexane was added to the mixture for the extraction. The funnel was shaken vigorously and allowed to stand for 15 min to enable separation of the organic solvent and aqueous layer. The n-hexane fraction was thereafter separated from the aqueous layer and collected in a clean flask. The extraction was done thrice until the layer became colourless. The n-hexane fractions collected were bulked and concentrated to dryness *in vacuo* using rotary evaporator. The n-hexane fraction was coded **HF** and was kept in the freezer for further use. The aqueous layer left was re-concentrated *in vacuo* to eliminate residual n-hexane. The same procedure described above was performed sequentially with the remaining solvents of chloroform (**CF**), diethyl ether (**DEF**), and butanol (**BF**).

2.0.6 Accelerated Gradient Chromatography of active fractions of the extract

The antimicrobially active diethyl ether

fraction (**DEF**) and butanol fraction (**BF**) were separately dissolved in 50 ml methanol, absorbed on silica gel₂₃₀₋₄₀₀ mesh and evaporated to dryness in a rotary evaporator. They were separately chromatographed on a silica gel column (650 $\diamond$ 40 mm) and gradiently eluted with a solvent system made from hexane, chloroform, methanol and ethyl acetate. The elution was started with n-hexane only and later continued with Chloroform/n-hexane (10:90), Chloroform/ethyl acetate (90:10), and finally Ethyl acetate/methanol (90:10), to obtain the diethy ether fraction **DEF_a** and **DEF_b**. For the butanol fraction, Chloroform was first used to elute the fraction and later followed by Chloroform/ethyl acetate (90:10), and finally ethyl acetate/ methanol (90:10). The elutes collected in test tubes were bulked, based on similarity shown on TLC plates, developed in a suitable solvent system and visualized under the UV-light at 254 and 366 nm. The plates were also sprayed with vanillin-sulphuric acid for colour development. The bulked fractions were concentrated to dryness *in vacuo* on a rotary evaporator and coded **DEF_a** and **DEF_b** for fractions collected from **DEF**. Fractions collected from **BF** were coded **BF_a** and **BF_b**.

2.0.7 Antibacterial activity assay

The antibiograms of the partitioned fractions **HF**, **CF**, **DEF** and **BF** were determined using the agar-well diffusion method as described by Irobi et al. (1994) and Russell and Furr (1977) with modifications. The bacterial isolates were first grown in nutrient broth for 18 h before use. Two hundred microliters of the broth culture was used to inoculate 20 ml of molten Mueller-Hinton agar (Oxoid) at 50 °C and then poured onto sterile Petri dishes and allowed to set. Wells were then bored into the agar medium using a sterile 6 mm cork borer. The wells were filled with the extract fractions, taking care to avoid spillage on the surface of the medium. Controls were set up in parallel using the solvents that were used to reconstitute the extract. The plates were allowed to stand on the laboratory bench for between 1-2 h to allow proper diffusion of the fractions into

the medium before incubating the plates at 37°C for 24h. The plates were later observed for the zones of inhibition. The effects of the extract on the test bacteria were compared to those of the standard antibiotics, streptomycin and tetracycline at a concentration of 1 mg/ml each.

2.0.8 Determination of Minimum inhibitory concentrations (MIC)

The MICs of the active fractions of the extract were determined using the description of Akinpelu and Kolawole (2004). Two-fold dilutions of the plant extract were prepared and 2 ml of different concentration of the solution were added to 18 ml of pre-sterilized molten nutrient agar at a temperature of 40°C to a series of concentrations of between 10 and 0.040 mg/ml. The media were then poured into sterile Petri dishes and allowed to set. The surfaces of the media were allowed to dry before streaking with 18 h old cultures of the susceptible test bacteria. The plates were later incubated in an incubator at 37 °C for up to 72 h after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented bacterial growth.

2.0.9 Determination of Minimum bactericidal concentrations (MBCs)

The MBC of the active fractions of the extract were determined as described by Olorundare et al. (1992) with modifications. Samples were taken from plates with no visible growth in the MIC assay and subcultured onto freshly prepared nutrient agar medium and later incubated at 37°C for 48 h. The MBC was taken as the lowest concentration of the extract that completely inhibited bacterial growth.

2.0.10 Determination of the rate of killing and protein leakage

Assay for the rate of killing of representative test bacteria by the active fractions was determined in accordance with the method of Odenholt et al. (2001). One representative

each of Gram positive and Gram negative bacterial isolates were used viz., *Bacillus anthracis* (Gram positive) and *Escherichia coli* (Gram negative). The turbidity of an 18 h old broth culture of the test organism was standardized to approximately 10^8 cfu/ml. A 0.5ml volume of the standardized suspension was added to 4.5 ml of different concentrations of the fractions. These were held at room temperature and the killing rate was determined over a period of 2 h. Exactly 0.5 ml volume of each suspension was withdrawn at time intervals and transferred to 4.5 ml of nutrient broth recovery medium containing 3% "Tween 80" to neutralize the effects of the antimicrobial compound carry-overs from the test organisms. The suspension was then serially diluted and plated out for viable counts. The plates were later incubated at 37 °C for 48 h. The control plates contained the bacterial cells without the extract. The emergent bacterial colonies were counted and compared with the counts of the culture control. Protein leakage was determined in accordance with the method in our previous description (Aiyegoro et al., 2008) by treatment of various concentrations of the extracts (relative to MIC) with bacterial cells washed three times in physiological saline by centrifugation at 10,000 rpm for 10 min followed by re-suspension in physiological saline. At intervals, each suspension was centrifuged at 7000 rpm and the supernatant obtained was assayed for protein using the Bradford method (1976). The concentration of protein was estimated from the established standard curve obtained using bovine serum albumin (BSA). *Bacillus anthracis* (Gram positive) and *Escherichia coli* (Gram negative) were also used for this assay.

3.0 RESULTS

The results of our investigations showed that the crude methanolic extract of *Garcinia kola* along with the two partially purified fractions (**DEFa** and **BFa**) exhibited antimicrobial activities against some of the test bacterial isolates. The two solvents diethyl ether and butanol, which served as controls did not show any appreciable

antimicrobial activity. The crude extract exhibited antimicrobial activity at a test concentration of 20 mg/ml while the two partially purified fractions exhibited activities at a lower concentration of 10 mg/ml (Tables 1 and 2). The lowest zone of inhibition exhibited by the crude methanolic extract of *G. kola* was 10 mm while the highest zone of inhibition was 23 mm against *E. coli*. On the other hand, fraction **DEFa** exhibited the highest zone of inhibition of 22mm against *B. stearothersophilus*, *Corynebacterium pyogenes*, and *M. luteus*. The lowest zone of inhibition exhibited by this fraction **DEFa** was 10 mm against *B. cereus*. The highest zone of inhibition exhibited by fraction **BFa** was 25 mm against *B. anthracis*, while the lowest zone of inhibition was 12 mm shown against *Enterococcus faecalis*. Fractions **HF** and **CF** did not inhibit the growth of any of the test bacteria. The two standard antibiotics, streptomycin and tetracycline used in this work exhibited zones of inhibition of 18 mm to 30 mm and 10 mm to 28 mm respectively.

The minimum inhibitory concentrations (MIC) of the fractions **DEFa** and **BFa** obtained from the crude extract of *G. kola* seeds along with that of streptomycin were also determined. The MIC of fraction **DEFa** varied between 0.313 and 5.0 mg/ml, while that of **BFa** varied between 0.157 and 5.0 mg/ml, and that of streptomycin was between 0.0157 and 0.125 mg/ml. The results indicated that streptomycin has stronger antimicrobial activity than the two fractions as indicated in Table 3.

Different concentrations of **BFa** fraction exhibited significant bactericidal effects on *E. coli* and *B. anthracis* over a period of time ranging between 15-120 mins. At a concentration of 1.25 mg/ml the **BFa** fraction killed about 19 % of *E. coli* cells within 30 min and the proportion of cells killed with time continue to increase with concentration of the extract to attain peak values of between 57 % and 100 % in 2 hr (Figure 1a). A similar trend of killing rate was observed for *Bacillus anthracis* (Figure 1b). The **DEFa** fraction also exerted bactericidal effect on the test organisms. For this fraction, between 42 % and 100 %

TABLE 1

Sensitivity patterns of some bacterial isolates to crude methanolic extract of *Garcinia kola* seeds

Microorganism	Zone of Inhibition (mm*)		
	<i>G. kola</i> extract (20 mg/ml)	Streptomycin (1 mg/ml)	Tetracycline (1 mg/ml)
<i>Bacillus anthracis</i> (LIO)	18	18	25
<i>Bacillus cereus</i> (NCIB 6349)	Nil	22	18
<i>Bacillus stearothermophilus</i> (NCIB 8222)	20	23	22
<i>Bacillus polymyxa</i> (LIO)	22	18	20
<i>Bacillus subtilis</i> (NCIB 3610)	20	23	22
<i>Clostridium sporogenes</i> (NCIB 532)	21	25	20
<i>Corynebacterium pyogenes</i> (LIO)	10	20	20
<i>Enterococcus faecalis</i> (NCIB 775)	10	23	28
<i>Escherichia coli</i> (NCIB 86)	23	Nil	18
<i>Klebsiella pneumoniae</i> (NCIB 418)	22	Nil	12
<i>Micrococcus luteus</i> (NCIB 196)	13	18	22
<i>Pseudomonas aeruginosa</i> (NCIB 950)	Nil	Nil	Nil
<i>Pseudomonas fluorescens</i> (NCIB 3756)	Nil	30	Nil
<i>Staphylococcus aureus</i> (NCIB 8588)	Nil	21	Nil
<i>Staphylococcus epidermidis</i> (LIO)	10	21	10

Key: NCIB = National Collection of Industrial Bacteria; LIO = Locally isolated organisms; mm* = Mean of three replicates.

TABLE 2

Sensitivity pattern of some bacterial isolates to various fractions of *Garcinia kola* seeds

Microorganism	Zone of inhibition (mm*)									
	BF	DEF	HF	CF	BC	DC	HC	CC	STR	TET
<i>Bacillus anthracis</i> (LIO)	25	22	0	0	0	0	0	0	18	25
<i>Bacillus cereus</i> (NCIB 6349)	14	10	0	0	0	0	0	0	22	18
<i>B. stearothermophilus</i> (NCIB 8222)	22	18	0	0	5	0	0	0	23	22
<i>Bacillus polymyxa</i> (LIO)	15	20	0	0	0	5	0	0	18	20
<i>Bacillus subtilis</i> (NCIB 3610)	20	20	0	0	5	5	0	0	23	22
<i>Clostridium sporogenes</i> (NCIB 532)	0	0	0	0	0	0	0	0	25	20
<i>Corynebacterium pyogenes</i> (LIO)	22	16	0	0	0	0	0	0	20	20
<i>Enterococcus faecalis</i> (NCIB 775)	12	0	0	0	0	0	0	0	23	28
<i>Escherichia coli</i> (NCIB 86)	21	18	0	0	0	0	0	0	0	18
<i>Klebsiella pneumoniae</i> (NCIB 418)	16	16	0	0	0	0	0	0	0	12
<i>Micrococcus luteus</i> (NCIB 196)	22	16	10	10	5	0	0	0	18	22
<i>Pseudomonas aeruginosa</i> (NCIB 950)	0	14	0	0	0	0	0	0	0	0
<i>Ps. fluorescens</i> (NCIB 3756)	17	0	0	0	0	0	0	0	30	0
<i>Staphylococcus aureus</i> (NCIB 8588)	0	0	0	0	0	0	0	0	21	0
<i>Staphylococcus epidermidis</i> (LIO)	0	0	0	0	0	0	0	0	21	10

Key: NCIB = National Collection of Industrial Bacteria; LIO = Locally Isolated organisms; 0 = Not sensitive; mm* = Mean of three replicates; DEF = Diethyl-ether Fraction (10 mg/ml); BF = Butanol Fraction (10 mg/ml); HF = Hexane Fraction (10 mg/ml); CF = Chloroform Fraction (10 mg/ml); BC = Butanol control; DC = diethyl ether control; HC = hexane control; CC = chloroform control; STR = Streptomycin (1 mg/ml); TET = Tetracycline (1 mg/ml).

TABLE 3

The minimum inhibitory concentrations (MIC) of active partially purified fractions and streptomycin

Microorganism	Fractions		
	DEFa (10 mg/ml)	BFa (10 mg/ml)	Streptomycin (1 mg/ml)
<i>Bacillus anthracis</i> (LIO)	0.313	0.157	0.125
<i>Bacillus cereus</i> (NCIB 6349)	5.0	2.5	0.0625
<i>Bacillus stearothermophilus</i> (NCIB 8222)	0.625	0.313	0.0313
<i>Bacillus polymyxa</i> (LIO)	1.25	2.5	0.125
<i>Bacillus subtilis</i> (NCIB 3610)	0.625	0.313	0.125
<i>Clostridium sporogenes</i> (NCIB 532)	ND	ND	0.0313
<i>Corynebacterium pyogenes</i> (LIO)	1.25	0.313	0.125
<i>Enterococcus faecalis</i> (NCIB 775)	ND	5.0	0.0313
<i>Escherichia coli</i> (NCIB 86)	1.25	0.625	ND
<i>Klebsiella pneumoniae</i> (NCIB 418)	1.25	1.25	ND
<i>Micrococcus luteus</i> (NCIB 196)	1.25	0.313	0.125
<i>Pseudomonas aeruginosa</i> (NCIB 950)	ND	ND	ND
<i>Pseudomonas fluorescens</i> (NCIB 3756)	2.5	1.25	0.0157
<i>Staphylococcus aureus</i> (NCIB 8588)	ND	ND	0.0625
<i>Staphylococcus epidermidis</i> (LIO)	ND	ND	0.0625

Key: DEFa = Diethyl-ether Fraction; BFa = Butanol Fraction; ND = Not Done.

of the *E. coli* cells were killed within 2 hr, with the proportion of killed cells increasing with extract concentration (Figure 1c). As well, the proportion of cells of *B. anthracis* killed ranged from 32 % for the lowest extract concentration of 0.652 mg/ml to 100 % for the highest extract concentration of 5 mg/ml (Figure 1d).

Both **BFa** and **DEFa** fractions caused considerable leakage of proteins from the cells of *E. coli* and *B. anthracis*. When *B. anthracis* cells were exposed to **BFa** fraction, between 8 µg/ml and 58 µg/ml protein were leaked from the cells in approximately 3 hr with the concentration of leaked protein increasing proportional to the concentrations of the **BFa** (Figure 2a). With *E. coli* cells, protein leaked within the same period ranged from 20 µg/ml to about 80 µg/ml (Figure 2b).

4.0 DISCUSSION

The antimicrobial activity of *G. kola* seeds extract successfully inhibited the growth of

both Gram-positive and Gram-negative organisms used in this study, thus, exhibiting a broad spectrum of activity. Fractions **HF** and **CF** used in our investigations did not exhibit antimicrobial activities against the tested isolates, unlike **DEFa** and **BFa**, which were active. This observation might be attributed to the fact that the active constituents of *G. kola* seeds are either moderately polar or polar in nature, since only polar solvent fractions of the extract were observed to be active. This might serve as a pointer to the type of organic solvent that can be used in extracting active ingredients of this plant. In addition, using the MIC values, there was a good comparison between **BFa** fraction and the standard antibiotics used in this study (Table 3). On the other hand, **BFa** fraction showed better activities than **DEFa** fraction. This might be attributed to the higher polarity of butanol and thus more affinity for the active components of *G. kola* than diethyl ether.

Overall, the *G. kola* extract compared favourably with the two standard

antibiotics. From this study, *G. kola* extract inhibited the growth of some of the pathogens tested and these include *E. coli* known to cause diarrhoea and urinary tract infections, as well as *Kleb. pneumoniae*, which is known to cause pneumonia and *Bacillus polymyxa*, the causative agent of bacterial seedling blight of tomato. These abilities of the plant extract to inhibit or kill these pathogens at minimal contact time and low concentration could be used in preventing the establishment of infections

by these pathogens, thus supporting the use of *G. kola* seeds for the treatment of the diseases caused by these pathogens.

Protein leakage from the *B. anthracis* and *E. coli* cells on exposure to different concentrations of fractions **BFa** and **DEFa** followed a classical monophasic effects. Oloke (1989) reported a similar observation for a different plant, *Aframomum melegueta*. The monophasic effect is the increase in leakage with increases in concentrations of the fractions. The **DEFa**

Figure 1: Profiles of rate of killing of *Escherichia coli* by **BFa** fraction (a); *Bacillus anthracis* by **BFa** fraction (b) fraction; *Escherichia coli* by **DEFa** fraction (c); and *Bacillus anthracis* by **DEFa** fraction (d) of *Garcinia kola* seeds.

fraction showed similar activities when the two test organisms were subjected to the effect of the fraction. With longer duration of exposure of these cells to the effect of **DEFa**, cell viability decreased with a more significant increase in protein leakage (Figure 2b). Thus, when susceptible bacteria are exposed to antimicrobial agents this results in the leakage of intracellular materials because of the damage caused in the membrane of such cells. Previous workers (Slamenova et al. (2002) and Aherne et al. (2007) have reported leakage of intracellular materials from some cells. Aherne et al. (2007) investigated the effect of phytochemical compounds in inducement of stress in test organisms leading to the disruption of the cell membrane and thus causing leakage of cellular materials, including proteins. The fraction **DEFa** and **BFa** used in this study were found to cause leakage in protein, which led to the cell death, as shown in Figures 2a and b. The leakage caused by the two fractions of *G. kola* extract is probably similar to that observed by these authors, thus suggesting cell membrane disruption and a probable mechanism of action of our **DEFa** and **BFa** extracts, more so with the presence of tannins in the extract, which have been

known to disrupt cell membranes (Scalbert, 1991).

The bactericidal activities of the fractions of *G. kola* were determined using viability studies. As the concentration of the fractions increased, the microbial population decreased as indicated in Figures 1a-d. There appears to be a relationship between the amount of cell constituents released and the number of organisms killed. For example, when the cells of *E. coli* were exposed to 1.25 mg/ml of the **BFa** fraction, about 19 % of the *E. coli* cells were killed (Figure 1a). During the same time interval, 8 % of the leakage of protein was caused by the **BFa** fraction (Figure 2b). Our investigations thus suggest that membrane components are the primary target of these two fractions (**BFa** and **DEFa**) of *G. kola* seed extract. With this action we concluded that disruption of the cell membrane by these fractions ultimately resulted in bacterial death as previously suggested by Cao et al. (2002). Hence, a probable mechanism of action of these fractions obtained from *G. kola* seed extract are by way of cell membrane disruption to *E. coli* and *B. anthracis* cells, although there might be other mechanism(s) of action exerted by these fractions that were not studied in our investigations.

Figure 2: The effect of partially purified **BFa** fraction of *Garcinia kola* on protein leakage in *Bacillus anthracis* (LIO) (a) and **DEFa** fraction of *Garcinia kola* on protein leakage in *E. coli* (NCIB 86) (b).

The phytochemical analysis of the extract of *G. kola* revealed the presence of flavonoids, tannins, cardiac glycoside, steroids, saponins and reducing sugars. These phytochemical compounds are known to play important roles in bioactivity of medicinal plants. The medicinal values of medicinal plants lie in these phytochemical compounds, and as such, produce definite physiological actions on the human body. Flavonoids, which are part of the phytochemical constituents of *G. kola*, exhibit a wide range of biological activities, one of which is their ability to scavenge for hydroxyl radicals, and superoxide anion radicals, and thus health promoting in action (Ferguson, 2001). Flavonoids also exhibit anti-inflammatory, anti-angionic, anti-allergic effects, analgesic and antioxidant properties (Hodek et al., 2002). These observations support the usefulness of *G. kola* in folklore remedies for the treatment of various infections. Cardiac glycosides are an important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure (Ikeda et al., 1995). This compound has been reported to be a novel cancer therapeutic agent (Newman et al., 2008). Cardiac glycoside was present in *G. kola* extract, and this plant is used for the treatment of cardiac infections, along with other ailments, such as cough and chest pain among Yoruba tribe of south-western Nigeria. Another phytochemical compound observed to be present in *G. kola* seeds extract is tannins. Tannins exert antimicrobial activities by iron deprivation, hydrogen binding or specific interactions with vital proteins, such as enzymes in microbial cells (Scalbert, 1991). Herbs that have tannins are astringent in nature and are used for the treatment of intestinal disorders, such as diarrhoea and dysentery (Dharmananda, 2003), and further supports why *G. kola* is among the medicinal plants used for the treatment of microbial infections. Tannins have been observed to have remarkable activity in cancer prevention (Li et al., 2003). In addition to this, Motal et al. (1985) showed tannins to be useful in treatment of inflamed or ulcerated tissues. Thus, the presence of

tannins in *G. kola* supports the traditional medicinal use of this plant in the treatment of ailments caused by microorganisms. Just et al. (1998) revealed inhibitory effect of saponins on inflamed cells. Saponin was also present in *G. kola* seed extract and has supported the usefulness of this plant in managing inflammation. Steroidal compounds also present in *G. kola* extract are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001). We conclude that *Garcinia kola* seeds could be a potential source of active antimicrobial agents, and a detailed assessment of their *in vivo* potencies is the subject of current extensive investigation by our group.

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