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Full Length Research Paper

The bioactive and phytochemical properties of *GARCINIA KOLA* (Heckel) seed extract on some pathogens

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***IN VITRO* antimicrobial activities of crude extract of *GARCINIA KOLA* was investigated against some bacterial isolates comprising of both Gram-positive and Gram-negative organisms. The methanolic crude extract exhibited significant inhibitory action against eleven out of fifteen bacterial isolates tested at a final concentration of 20 mg/ml. The zones of inhibition exhibited by the extract against the tested organisms ranged between 10 and 23 mm, while the zones of inhibition exhibited by streptomycin and tetracycline used as standard antibiotics ranged between 15 and 25 mm; 12 and 25 mm respectively. On the other hand, the minimum inhibitory concentrations exerted by the extract against the bacterial isolates ranged between 0.079 and 5.00 mg/ml while the ranged exhibited by streptomycin was between 0.0157 and 0.50 mg/ml. The plant extract compared favourably with the two standard antibiotics used in this study. The following phytochemical compounds were present in the plant extract: flavonoids, tannins, cardiac glycoside, saponins, steroids and reducing sugars.**

Key words: *Garcinia kola*, antimicrobial activity, minimum inhibitory concentrations, phytochemical compounds.

INTRODUCTION

The study of medicinal plants used in folklore remedies in the treatment of microbial infections have attracted the attention of many scientists as possible alternatives to the existing drugs to which many infectious microorganisms have become resistant. Presently, there are global problems of multiple antibiotics resistance as well as emergence of new and resurrection of previously eradica-ted diseases. Reports on ethnobotanical records indicate a general consensus on the use of antimicrobially active medicinal plants to provide cheaper drugs. There is need to search for new and more potent antimicrobial compounds of natural origin to complement the existing synthetic antimicrobial drugs that are gradually becoming less potent against pathogenic microorganisms. This study therefore focuses on the antimicrobial potency of *Garcinia kola*. *G. kola* belongs to the family Guittiferae and it is commonly called “Orogbo” in Yoruba language while the English name is bitter kola. The plant has been referred to as a “wonder plant” 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 also used to prevent and relieve colic, chest colds, cough and can as well be used 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 also found usefulness in the treatment of stomach ache and gastritis (Ajebesone and Aina, 2004). The phytochemical compounds isolated from *G. kola* include oleoresin (Onayade et al., 1998), tannins, saponins, alkaloids, cardiac glycosides (Ebana et al., 1994). Other phytochemical compounds so far isolated from *G. kola* seeds are biflavonoids such as kolaflavone and 2-hydroxybi-flavonols (Okunji and Iwu, 1991; Terashima et al., 1999; Okunji et al., 2002). Two new chromanols, garcioic and garcinal, together with δ=tocotrienol were reported isolated from *G. kola* (Terashima et al., 2002). The biflavanones are predo-minant compounds in *G. kola* and kolaflavanones are major components of kolaviron (Iwu, 1985). *G. kola* is used in folklore remedies for the treatment of various infections caused by pathogens. This study therefore focused on the bioactive potentials of the extract from the plant on some microorganisms.

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MATERIALS AND METHODS

Plant material

Fresh seeds of *G. kola* were purchased from a market in Ile Ife, Osun State, Nigeria. They were authenticated at the Herbarium of Botany Department, Obafemi Awolowo University, Ile Ife, Nigeria. Voucher specimen was deposited at the Herbarium for reference. The seeds were peeled and cut into pieces and later dried in the hot-air oven at 40^0C until the constant weight of the seeds was obtained. The seeds were later powdered and kept in an air-tight container for further use.

Preparation of the plant extract

Exactly 1200 g of the powdered seeds was soaked in mixture of methanol and sterile distilled water in ratio 3:2 for four days and later filtered to obtain the methanolic extract. The mixture was first concentrated *in vacuo* using rotary evaporator to remove the methanol. The aqueous residue was later lyophilized to get the crude extract which was dark brown in colour. The yield collected was 250 g.

Preparation of microorganisms for the experiment

The following typed cultures and locally isolated organisms obtained from culture collection of the Microbiology Department, Obafemi Awolowo University, Ile Ife, Nigeria were used for the experiment. These bacterial isolates include Gram-positive: *Bacillus subtilis* (NCIB 3610), *Staphylococcus aureus* (NCIB 8588), *Streptococcus faecalis* (NCIB 775), *Micrococcus luteus* (NCIB 196), *Bacillus cereus* (NCIB 6349), *Bacillus stearothermophillus* (NCIB 8222), *Bacillus polymyxa* (LIO), *Bacillus anthracis* (LIO), *Staphylococcus epidermidis* (LIO), *Clostridium sporogenes* (NCIB 532), *Corynebacterium pyogenes* (LIO). The Gram-negative bacteria were *Escherichia coli* (NCIB 86), *Pseudomonas aeruginosa* (NCIB 950), *Pseudomonas fluorescens* (NCIB 3756), *Klebsiella pneumoiae* (NCIB 418). For the experiments, the bacterial isolates were first subcultured in nutrient broth (Oxoid, Ltd.) and incubated at 37^0C for 18 h.

Phytochemical screening test for the extract

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

Test for alkaloids

Exactly 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl on steam bath. A millilitre of the filtrate was treated with drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloids.

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.

Test for flavonoids

A 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.

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 as negative control while commercial saponin (BDH) solution was used as positive control. The plates were incubated at 35^0C for 6 h. Complete haemolysis of the blood around the extract was indicative of saponins.

Test for steroids

Salkowski method 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.

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 spreads throughout this layer.

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 min. The production of a brick red precipitate indicated the presence of reducing sugars.

Sensitivity testing of *G. KOLA* extract on bacterial isolates

The sensitivity testing of the plant extract was determined using agar-well diffusion method as described by irobi et al. (1994), Russell and Furr (1977) with little modifications. The bacterial isolates were first grown in nutrient broth for 18 h before use. The isolates were later subcultured on to Mueller-Hinton agar (Oxoid, Ltd.). Wells were then bored into the agar medium using a sterile 6 mm cork borer. The wells were then filled up with the solution of the extract and care was taken not to allow the solution to spill to the surface of the medium. The plates were allowed to stand on the laboratory bench for between 1 – 2 h to allow proper inflow of the solution into the medium before incubating the plates in an incubator at 37^0C for 24 h. The plates were later observed for the zones of inhibition. The effects of the extract on bacterial isolates were compared with those of standard antibiotics, streptomycin and tetracycline at a concentration of 1 mg/ml each.

Minimum inhibitory concentrations (MIC) of the extract on the bacterial isolates

The MIC of the extract was determined using method of Akinpelu and Kolawole (2004). Two-fold dilutions of the plant extract was

Table 1. Sensitivity patterns of zones of inhibition exhibited by the crude extract of *G. kola* on some pathogens.

Microorganism	Zone of inhibition (mm*)		
	G. KOLA extract (20 mg/ml)	Streptomycin (1 mg/ml)	Tetracycline (1 mg/ml)
Bacillus anthracis (LIO)	18	18	25
Bacillus cereus (NCIB 6349)	0	15	18
Bacillus stearothermophilus (NCIB 8222)	20	23	22
Bacillus polymyxa (LIO)	22	15	20
Bacillus subtilis (NCIB 3610)	20	23	22
Clostridium sporogenes (NCIB 532)	21	25	20
Corynebacterium pyogenes (LIO)	10	20	20
Escherichia coli (NCIB 86)	23	0	18
Klebsiella pneumoniae (NCIB 418)	22	0	12
Micrococcus luteus (NCIB 196)	13	25	22
Pseudomonas aeruginosa (NCIB 950)	0	21	0
Pseudomonas fluorescens (NCIB 3756)	0	25	0
Staphylococcus aureus (NCIB 8588)	0	21	0
Staphylococcus epidermidis (LIO)	10	21	10
Streptococcus faecalis (NCIB 775)	10	23	28

NCIB = National Collection of Industrial Bacteria; LIO = locally Isolated organisms; 0 = resistant; mm* = Mean of three replicates in mm.

prepared and 2 ml of different concentration of the solution was added to 18 ml of pre-sterilized molten nutrient agar at temperature of 40^0C to give final concentrations of 10, 5, 2.5, 1.25, 0.625, 0.313, 0.157, 0.079 and 0.040 mg/ml. The medium was then poured into sterile Petri dishes and allowed to set. The surface of the medium was allowed to dry before streaking with 18 h old isolates. The plates were later incubated in an incubator at 37^0C 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 will prevent the bacterial growth.

Minimum bactericidal concentrations (MBC) of the plant extract on bacterial isolates

The MBC of the extract was determined using Olorundare et al. (1992) method with little modifications. Samples were taken from plates with no visible growth in the MIC assay and subcultured on to freshly prepared nutrient agar medium and later incubated at 37^0C for 48 h. The MBC was taken as the lowest concentration of the extract that did not allow any bacterial growth on the surface of the agar plates.

RESULTS AND DISCUSSION

The investigations done on *Garcinia kola* extract revealed that the plant possesses antimicrobial activities against the tested bacterial isolates at a final concentration of 20 mg/ml (Table 1). The extract exhibited activities against eleven out of fifteen bacterial isolates comprising of both Gram-positive and Gram-negative organisms. The results thus shows that the extract possess a broad spectrum activities. *S. epidermidis* and *S. faecalis* show the least zone of inhibition of 10 mm while *E. coli* has the highest zone of inhibition of 23 mm. On the other hand zones of inhibitions exhibited by streptomycin one of the standard antibiotics used ranged between 15 and 25 mm. From this observation, *G. kola* extract compared favourably with the standard antibiotic, streptomycin. The MIC of the plant extract against the tested bacterial isolates was also determined. The MIC varied between 0.079 and 5.00 mg/ml. The standard antibiotic, streptomycin had MIC values varying between 0.0157 and 0.5 mg/ml. The results indicated that the standard antibiotic has stronger activity than the crude extract of *G. kola* as shown in Table 2. From all indications, if the crude extract becomes more purified the activities might look stronger than that of the crude extract and has almost the same activity with the standard antibiotic. This will form part of our next investigations.The phytochemical analysis of the extract of *G. kola* revealed the presence of flavonoids, tannins, cardiac glycoside, steroids, saponins and reducing sugars (Table 3). These phytochemical compounds are known to play important roles in bioactivity of medicinal plants. The medicinal values of medicinal plants lies in these phytochemical compounds and as such produce a 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 scanvenge 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

Table 2. The minimum inhibitory concentrations (MIC) exhibited by the extract against the bacterial isolates.

Microorganism	*GARCINIA KOLA* (mg/ml)	Streptomycin (mg/ml)
Bacillus anthracis (LIO)	0.625	0.0313
Bacillus cereus (NCIB 6349)	1.25	0.0313
Bacillus stearothermophilus (NCIB 8222)	0.079	0.0157
Bacillus polymyxa (LIO)	1.25	0.0625
Bacillus subtilis (NCIB 3610)	1.25	0.0625
Clostridium sporogenes (NCIB 532)	1.25	0.0625
Corynebacterium pyogenes (LIO)	0.313	0.0313
Escherichia coli (NCIB 86)	0.157	-
Klebsiella pneumoniae (NCIB 418)	0.079	-
Micrococcus luteus (NCIB 196)	0.157	0.0625
Staphylococcus epidermidis (LIO)	0.313	0.50
Streptococcus faecalis (NCIB 775)	5.00	0.0625

NCIB = National Collection of Industrial Bacteria; LIO = locally Isolated organisms.

Table 3. Phytochemical compounds present in *Garcinia kola* crude extract.

Phytochemical compound	Result
Alkaloids	Negative
Steroids	Positive
Cardiac glycosides	Positive
Flavonoids	Positive
Tannins	Positive
Saponins	Positive
Reducing sugar	Positive

are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure (Yukari et al., 1995). This compound has been reported to be a novel cancer therapeutic agent (Robert 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 chestpain among Yoruba tribe of southwestern Nigeria. Another phytochemical compound observsed to be present in *G. kola* extract is tannins. Tannins exert antimicrobial activities by iron deprivation, hydrogen bounding or specific interactions with vital proteins such as enzymes in microbial cells (Scalbert, 1991). Herbs that have tannins as their component are astringent in nature and are used for the treatment of intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003), this finding support the reasons why *G. kola* has position among medicinal plants used for the treatment of microbial infection. Tannins has been observed to have remarkable activity in cancer prevention and anticancer (Li et. al., 2003). In addition to this Motar et al. (1985) showed tannins to be useful in treatment of inflamed or ulcerated tissues. Thus, the presences of tannins in *G. kola* support 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* extract and has supported the usefulness of this plant in managing inflammation. Steroidal compound also present in *G. kola* extract are of importance and interest due to their relationship with such compounds as sex hormone (Okwu, 2001).*G. kola* ranked well among the medicinal plants used routinely among many tribes in Nigeria and some part of Africa for the treatment of infections caused by microorganisms. Apart from been used for folklore remedies, *G. kola* seeds are also chewed by many people because of their bitter taste. These suggest that the seeds are not toxic and hence there is need for the preparation of different formulations towards ensuring acceptable dosing regime pursuant to clinical trials. Further work will be carried out on the mechanisms of action of the plant.

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