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Full Length Research Paper

In vitro antibacterial activity of crude ethanol, acetone and aqueous *Garcinia kola* seed extracts on selected clinical isolates

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The study was conducted to screen for *in vitro* antibacterial activity of crude ethanol, acetone and aqueous seeds extract of *Garcinia kola* at different treatment regimes against some selected clinical bacterial isolates comprising of Gram positive and negative organisms namely; *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* and the major chemical groups responsible for the activity were determined. The agar well diffusion method was employed to determine the inhibitory effects of the seeds extract on the test microorganisms. The minimum inhibitory concentration exerted by the extracts against the bacterial isolates ranged between 3.125 and 25 mg/ml. The zones of inhibition exhibited by the extracts against the tested bacterial isolates ranged between 4.0 and 10.5 mm. The crude ethanol extract was found to exhibit more significant (P<0.01) inhibitory action against all the bacterial isolates at the various treatment regime. Also, compared to crude acetone and aqueous extracts, it was also notably found to exhibit significant (P<0.05) effects against the bacterial isolates. The preliminary phytochemical test revealed the presence of flavonoids, tannins, saponins, sterols and terpenes as the major chemical groups in the plant extracts. The results of this study revealed that the *in vitro* antibacterial activity exhibited by the seeds extract may be attributed to the presence of these phytochemical compounds.

Key words: *In vitro* antibacterial activity, phytochemical, *Garcinia kola* seeds, bacterial isolates.

INTRODUCTION

Garcinia kola popularly known as bitter kola is one of the useful indigeneous tree in Nigeria and in West and Central Africa (Anegbeh et al., 2006). It is known as 'Orogbo' in Yoruba land, 'Namijin-goro' among the Hausas, 'Akuilu' in Igbo land and 'Zhila-goro' in Zalidva. It is cultivated and distributed throughout West and Central Africa and is known mostly for its antimicrobial potentials (Natural Standard Monograph, 2008).

The seed of *G. kola* is believed to possess many useful medicinal properties (Esimone et al., 2007). The regions of the plant of immense medicinal value are the roots, barks, stems, leaves and seeds to the use of extract and decoctions from the plant (Sofowora, 1982; Nwanguma, 1999; Ogbulie et al., 2004). The seeds of *G. kola* have pharmacological uses in the treatment of numerous infections and diseases associated with both man and animals such as cough, throat infections, bronchitis, hepatitis, liver disorders and stomach upset (Farombi et al., 2005; Hassain et al., 1982; Iwu and Igboko, 1986; Middleton and Kandaswani, 1991; Iwu, 1999; Meserole, 1999). Moreover, *G. kola* is highly recommended in the treatment of HIV and AIDS because of its antiviral, detoxification and cleansing properties (Oguntola, 2008).

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MATERIALS AND METHODS

The plant sample was purchased from Wunti market at Bauchi Town in Northern Nigeria. They were purchased fresh and packed in clean polythene bags and transported to the herbarium for identification. The identity of the plant was finally authenticated at

the Herbarium of Federal College of Forestry Jos, Plateau State, where the specimens were deposited.

Test organisms

Clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* were obtained from the Department of Microbiology of State Specialist Hospital Bauchi. The isolates were re-identified and subcultured on nutrient agar slants and stored at 4°C until needed for the analysis.

Preparation of the seeds extracts

The seeds were dried under room temperature and then ground into fine powder using mortar and pestle. The powdered seeds were extracted using soxhlet method of extraction with diethyl ether (Obi and Onuaha, 2000) and cold extraction method with water (Akerele et al., 2007). The extracts were reduced to dryness in a rotary evaporator at 50°C and the dried extracts were stored in air tight colored bottle.

Antibacterial testing

The agar well diffusion method as described by Ntiejumokwu and Alemika (1991) and Ogueke et al. (2006) was used to determine the inhibitory effects of the seeds extracts against the isolates. The bacterial isolates were first grown in nutrient broth for 18 h at 37°C, then 0.2 ml of the broth culture of the isolates were aseptically inoculated onto a molten nutrient agar which had been cooled to 45°C, mixed gently and poured into sterile petridishes and allowed to set. The extracts were tested at 50, 100 and 150 mg/ml concentration. These were delivered into wells (6 mm diameter) bored unto the surface of the inoculated nutrient agar plates. The extracts were allowed to diffused into the medium for 30 min. The plates were incubated at 37°C for 24 to 48 h. The zones of inhibition were measured in millimeter diameter using meter rule (Adegboye et al., 2008).

Determination of minimum inhibitory concentrations (MICs)

The minimum inhibitory concentrations of the extracts was determined using the agar dilution method of Akinpelu and Kolawole (2004), Irobi et al. (1994), Russell and Fur (1977) and Akerele et al. (2007). The extracts were incorporated into the growth medium at concentration of 150, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/ml. The plates were incubated at 37°C for 72 h after about 30 min of inoculation. The minimum inhibitory concentration was taken as the lowest concentration of the plant extract.

Phytochemical screening test for the plant extracts

A portion of the plant sample was subjected to phytochemical screening to test for tannins, flavonoids, saponins, alkaloids, sterols and terpenes using the methods described by Trease and Evan (1989) and Sofowora (1993).

Test for tannins

3 g of the powdered sample was bolied in 50 ml of sterile distilled water for 30 min and was filtered. The resulting filtrate was used to carry out the test using ferric chloride ($Fecl_3$) method. A portion of the aqueous extract was diluted with sterile distilled water in a ratio of 1:4 and a few drop of 10% ferric chloride solution was added. A blue or green colour indicates the presence of tannins (Evan, 1989).

Test for flavonoids

Sodium hydroxide method was used to test for flavonoids. 5 g of the powdered sample was completely detanned with acetone and the acetone was evaporated on a water bath. The residue was extracted in warm water and filtered. 5 ml of 10% NaOH was added to an equal volume of the filtrate and a yellow colouration indicates the presence of flavonoids (Sofowora,1993).

Test for saponins

The froth method was used to carry out the test (Sofowora, 1993). Small quantity of the powdered sample was added to 95% ethanol and boiled. The mixture was then filtered and 2.5 ml of the filtrate was added to 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 s. It was then allowed to stand for over 30 min. Honey cumb Froth was indicative of the presence of saponins.

Test for alkaloids

0.5 g of the plant sample was dissolved in 5 ml of 1% HCL on steam bath. 1 ml of the filtrate was then treated with drops of Dragendorff's reagent and turbidity or precipitation was taken as indicative of the presence of alkaloids (Trease and Evans, 1983; Harbourne, 1983).

Test for terpenes and sterols

Firstly, 0.5 g of the powdered sample was extracted by maceration with 50 ml of 95% ethyl acetate, filtered and then the filtrate was evaporated to dryness. The residue was dissolved in 10 ml of anhydrous chloroform and filtered. The filtrate was divided into two equal portion and this was used to carry out the following test;

Terpenes

The Lichermann-Burchard method was used for the test. First portion of the chloroform was mixed with 1 ml of acetic anhydride and 1 ml of concentration H_2SO_4 down the wall of the test tube to form a layer underneath. The formation of a reddish violet colour was indicative of the presence of terpenes (Sofowora, 1993).

Sterols

The Salkowski's method was used to test for sterols. The second portion of the chloroform solution was mixed with 1 ml of concentration H_2SO_4 carefully to form a layer underneath. A reddish brown colour was indicative of the presence of a Steroidal ring (Sofowora, 1993).

Statistical analysis

Data were subjected to two ways analysis of variance (ANOVA) using the randomized complete block designs. The response of the bacterial isolates to the different extracts at various concentrations

Table 1. *In vitro* antibacterial activity of crude ethanol *Garcinia kola* seeds extract on selected clinical isolates.

Concentration of extract (mg/ml)	Zones of inhibition (mm)		
	Sa	Ec	Pa
50	6.5	6.0	4.6
100	8.5	8.0	6.3
150	10.5	9.5	8.0

Sa = *Staphylococcus aureus* ; Ec = *Escherichia coli* ; Pa = *Pseudomonas aeruginosa*.

Table 2. *In vitro* antibacterial activity of crude acetone *G. kola* seeds extract on selected clinical isolates.

Concentration of extract (mg/ml)	Zones of inhibition (mm)		
	Sa	Ec	Pa
50	4.0	3.5	3.5
100	6.5	5.5	4.5
150	8.5	6.5	7.5

Table 3. *In vitro* antibacterial activity of aqueous *G. kola* seeds extract on selected clinical isolates.

Concentration of extract (mg/ml)	Zones of inhibition(mm)		
	Sa	Ec	Pa
50	4.0	3.5	3.5
100	5.5	4.5	5.5
150	6.0	7.0	6.5

of the treatment regimes; 50, 100 and 150 mg/ml and the mean of the zones of inhibition were determined.

RESULTS

The results show that the plant extracts possessed strong antibacterial activities against the tested clinical bacterial isolates at the various treatment regimes; 50, 100 and 150 mg/ml as indicated in Tables 1 to 3. The result reveals that crude ethanol extract exerted significant activities ($P<0.01$) against all the tested bacterial isolates at the various treatment regimes with *S. aureus* having a wider zones of inhibition followed by *E. coli* and *P. aeruginosa* with the lowest inhibitory zones. The minimum inhibitory concentrations(MICs) of the crude ethanol extract against the isolates was 3.125, 6.25 and 12.5 mg/ml for *S. aureus, E. coli* and *P. aeruginosa* respectively as shown in Table 4.

The *in vitro* activity of crude acetone extract was also showed to exhibit inhibitory effects ($P<0.05$) at the various treatment regimes against *S. aureus, E. coli* and *P. aeruginosa* with MICs of 12.5, 25 and 25 mg/ml, respectively as shown in Table 4. The aqueous extract also indicated a significant ($P<0.05$) activity against the clinical isolates at the various treatment regimes with *E. coli* yielding the highest zones of inhibition followed by *P. aeruginosa* and *S. aureus* with MICs of 12.5, 12.5 and 25 mg/ml, respectively as shown in Table 4. More also, the phytochemical screening test revealed the presence of flavonoids (++), tannins (+), saponins (+), terpenes and sterols (+) and absence of alkaloids (-) as shown in Table 5.

DISCUSSION

This study reveals that crude ethanol, crude acetone and aqueous *G. kola* seed extracts possess *in vitro* antibacterial activities at varying concentration against the clinical isolates. This is in conformity with the findings as reported by Esimone et al. (2007) that the seeds of *G. kola* is believed to possess many medicinal properties which includes anti-inflammatory, antibacterial, anti-microbial, antiviral, antidiabetic, purgative, and antihe-patotoxic (Ebana et al., 1991; Iwu, 1993; Akoechere et al., 2004; Anegbeh et al., 2006). Muanya (2008) also identified *G. kola* to have strong antibiotic activities and

Table 4. minimum inhibitory concentrations of *G. kola* seeds extracts on selected clinical isolates (mg/ml).

Test organism	Crude ethanol	Crude acetone	Aqueous
S. aureus	3.125	12.5	12.5
E. coli	6.25	12.5	12.5
P.aeruginosa	12.5	25	25

Table 5. Phytochemical Screening test for the plant extracts.

Chemical group	Extract
Flavonoids	+ +
Saponins	+
Alkaloids	+
Tannins	-
Sterols &Terpenes	+

+ + = Present in abundance; + = Present in low concentration; - = absent.

found the plant to be very effective against disease-causing microorganisms such as *E. coli, S. aureus, P. aeruginosa, Salmonella spp., Streptococcus spp., Candida albicans, Vibrio cholera* and *Neisseria gonorrhoea*.

The crude ethanol extracts was found to exhibit the most significant ($P<0.01$) activity against the tested clinical isolates at the various treatment regimes. Hence, *S. aureus* yielded wider zone of inhibition than other isolates and *E. coli* prove better than its counterparts. This results agree with the findings as reported by Obi and Onuoha (2000) and Ogueke et al. (2006) that ethanol is the best solvent for the extraction of most plant active principles of medicinal properties. Also, PMID (2008) in their investigation conducted on 338 individuals with running nose, cough and catarrh found that ethanolic extracts of bitter kola exhibited antibacterial activities against the pathogens; *S. aureus, Streptococcus pyogenes, Streptococcus pneumoniae,* and *Haemophilus influenzae*.

Similarly, the response between the bacterial isolates was not significant ($P>0.05$).The mean treatment revealed that 150 mg/ml produces significantly higher diameter of zones of inhibition compared to 50 and 100 mg/ml concentration. The MICs of the crude acetone extract suggest the treatment to be 12.5, 12.5 and 25 mg/ml for *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively. This agrees with the findings as reported by Siband et al. (2008) on *in vitro* antibacterial regimes of crude aqueous and acetone *G. kola* seed extracts. It was drawn from their conclusion that acetone *G. kola* seeds extract possess strong bacteriocidal activity and chemotherapeutically useful in the treatment of bacterial infections in humans. Also, in this study it was shown that aqueous *G. kola* seeds extract against the bacterial isolates share the same effect on the diameter yield. The treatment regimes was found to be significant ($P<0.05$) which affect the diameter yield of the bacterial isolates. The mean proves *P. aeruginosa* to be the best with the highest zones of inhibition at the various treatment regimes. This is in consistency with the investigation of Ogbuhe et al. (2007) on the antimicobial efficacy of cold, hot water extracts and ethanol extract of *G. kola* seeds which revealed that cold and hot water extract of *G. Kola* seeds moderately inhibited the growth of *S. aureus* and *S. pyogenes* with zone of inhibition of between 9 to 15 mm.

The phytochemical screening of the plant extracts was also determined in this study. The phytochemical analysis of the *G. kola* seeds extract revealed the presence of flavonoids, tannins, saponins, sterols and terpenes (Table 5). These phytochemical compounds plays a significant role in the *in vitro* antibacterial activity of the plant lies in these phytochemical compounds which produces a definite and specific action on the human body (Adegboye et al., 2008). A preliminary phyto-chemical test conducted by Esimone et al. (2007) revealed that the most abundant phytoconstituent in *G. kola* seeds are flavonoids, tannins and alkaloids respectively. Other constituents are protein, glycoside, reducing sugar, starch, sterols and triterpenoids. Similarly, Sofowora (1974) have also observed the presence of such constituents as flavonoids, tannins, saponins, alkaloids and glycoside, some of which have been shown to exhibit varying antimicrobial biological activities. Flavonoids which are part of the phytochemical constituents of *G. kola* are known to have hypoglycemic activity used in the treatment of diabetes. Adegboye et al. (2008) also revealed in their findings that the anti-microbial properties of *G. kola* seeds are attributed to the flavonoids and benzophenones. Flavonoids (especially biflavonoids) have been found to be the most abundant phytoconstituents of *G. kola* seeds (Iwu and Igboko, 1982). Flavonoids also exhibit anti-inflammatory, anti-angionic, anti-allergic effect, analgestic and antioxidant properties (Hodek et al., 2002). Flavonoids exhibit a wide range of biological activities one of which is their ability to scavange for hydroxyl radicals and superoxide anion radicals and thus health promoting in action (Ferguson, 2001).

Tannins which are known for the treatment of ulcer (Adegboye et al., 2008) have been identified in this study.

Motar et al. (1985) also showed tannins to be useful in the treatment of inflammed or ulcerated tissues. Tannins has also been observed to have remarkable activity in cancer prevention and anticancer (Li et al., 2003). Dharmananda (2003) also revealed that herbs' tannins as their component are astringent in nature, are used for the treatment of intestinal disorder such as diarrhea and dysentry. Tannins also exert antimicrobial activities by non-deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells (Scalbert, 1991). Tannins when applied to the gastric mucosa in low concentration render the outer most layer permeable and most resistant to uritation (Cole, 1992). He also indicated that tannins could also induce local vaso-constriction in small mucous. This findings support the reason why *G. kola* has position among medicinal plants used for the treatment of mcrobial infection and ailments caused by micro-organisms.

Saponins, which also supported the importance of this plant in managing inflammation (Adegboye et al., 2008), have also been identified in this study. The chemical saponin is mainly used as toxics for the liver. It enhances the functions of the liver and gall bladder (Nwokeke, 2008). Just et al. (1998) revealed inhibiting effect of saponin inflammed cells. In addition, steroidal compound also present in *G. Kola* seeds extract, are of importance and of interest due to their relationship with such compounds as sex hormone (Okwu, 2001). The presence of steroids in plant material suggest the ones already known (Adegboye et al., 2008). It is possible that steroids occur as part of aglycone moieties of other constituents of plant like saponins and alkaloid (Harbone, 1983). The findings in this study justified the facts that *G. Kola* seeds is a wonder plant because almost every part of its has been found to be of medicinal importance (Oguntola, 2008). It also agreed with the facts that the seeds as a whole is shown to have both anti-flammatory, antidiabetic and antihepotoxic activity (Iwu, 1993) and therefore ranked well among the medicinal plants used routinely among many tribes in Nigeria and some parts of Africa for the treatment of infectous caused by microorganisms.

Conclusion

The study shows that the *in vitro* antibacterial activity of the seed extracts against the chemical isolates at the various treatment regimes may be attributed to the presence of these phytochemical compounds identified in this study. Moreover, ethanol extracts was found to exhibit most significant ($P<0.01$) activity against the tested clinical isolates in this study compared to other extracts which also proved to be significantly ($P<0.05$) effective. This may be due to the fact that ethanol was found to be the best solvent of extraction of the active principles of medicinal Importance in plant (Obi and Onuoha, 2000; Ogueke et al., 2006). The MICS of the *G. Kola* seed extracts varied between 3.125 and 25 mg/ml against all the tested clinical bacterial isolates in this study.

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