ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF ETHANOL EXTRACT OF *Garcinia kola* (seed)

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Background

Garcinia kola Heckel, often called bitter kola, is an indigenous medicinal tree belonging to the family *Guttiferae*. Phytochemical analysis of extracts from both root, stem, and seed of this plant, and other members of the genus show that they contain reasonable amounts of secondary metabolites, most prominent which are phenolic compounds including biflavonoids (GB-1, GB-2), xanthones and benzophenones (Onunkwo *et al*., 2004; Okunji *et al*., 2007). Interest in the use of plant and plant products in the management of ailments, has increased rapidly due to the widespread of antibiotic resistance and side effects of most conventional drugs used as antibiotics (Babayi *et al*., 2004). The setbacks in the use of conventional antibiotics, has call for wide investigation of the antimicrobial activities of plants having high potentials for antimicrobial properties like *Garcinia kola*, against pathogenic microorganisms with a view of identifying novel therapeutic properties, that could be exploited in the development of new antimicrobial agents against infectious diseases.

Objectives

- To extract *Garcinia kola* seed using ethanol as solvent.
- To qualitatively determine the phytochemicals present in the ethanol extract.
- To determine the zone of inhibition of the extract at different concentrations on some selected pathogenic microorganisms.
- To determine the minimum inhibitory concentration of the extract on the selected microorganisms**.**

Methods

Sample collection and processing

The seeds of *Garcinia kola* were obtained from dealers at the railway market, Minna, Niger state. The seeds *were* dehusked and then air-dried for a period of two weeks, after which they were grounded into fine particles using mortar and pestle.

Extraction procedure

Fine particles of the sample (100 g) was extracted with 99% ethanol (500ml) in the ratio of 1:5 (w/v) at room temperature for 2 days. The filtrate was evaporated to dryness at 70 ^{0}C, and stored in a freezer until needed for subsequent analysis.

Phytochemical screening of extract

Qualitative phytochemical screening to detect the presence of alkaloids, saponins, cardiac glycosides, steroids, terpenes, tannins, reducing sugars, flavonoids, phenols, and anthranoids was carried out on the extract using the methods of Trease and Evans (1989), and Sofowora (1993).

Test organisms.

Pure cultures bacteria and fungi isolates (*Salmonella typhimurium, Pseudomonas aeruginosa, E. coli, Aspergillus flavus, Aspergillus niger, and Candida albicans)* were obtained from the Department of Microbiology, School of Life Sciences, Federal University of Technology, Minna. Niger State.

Culture media

Nutrient agar, Sabouraud dextrose agar (SDA), and Nutrient broth prepared using standard laboratory procedures were used for the investigation.

Standardization of inoculum

This was done by sub-culturing about 4-5 colonies from the pure growth of each test organism and incubated at 37^{0}c for 24 hours. The turbidity of the culture was compared to McFarland Standard.

Agar well diffusion for antimicrobial susceptibility testing

Antimicrobial susceptibility test was evaluated using the agar-well diffusion method (Perez *et al.,* 1990). Nutrient agar plates for bacteria and SDA plates for fungi, were used to drill holes of diameter 4mm each using a sterile cork borer. Each hole was seeded and incubated with 0.2ml of the reconstituted extract of different concentrations; 100mg/ml, 200mg/ml and 300mg/ml. Standard antibiotics (0.2g) (ampiclox for bacterial isolates; Ketoconazole for fungal isolates) were used. The diameters of clear zones of inhibition were measured and recorded.

Determination of minimum inhibitory concentration (MIC)

Determination of MIC of the extract on the organisms was carried out using the tube dilution method (Olukemi *et al.,* 2004). 8 test tubes containing 8ml of sterile nutrient broth were use. Tube 1 was used as negative control. A serial dilution was carried out to a final concentrations of 40mg/ml, 8mg/ml, 1.6mg/ml, 0.32mg/ml, 0.064mg/ml and 0.0128mg/ml, 0.00256mg/ml. Each tube was added 10^8cfu/ml of different test organism. The tubes were incubated at 37^{0}c for 24 hours. The lowest dilution that showed no visible turbidity was regarded as minimum inhibitory concentration.

Results

Table 1: Qualitative Phytochemical Screening of Ethanol Extract of *Garcinia kola* Seed

Phytochemicals	Concentrations
Alkaloids	-
Saponins	+
Cardiac glycosides	+
Reducing sugars	+
Steroids	+
Terpenes	+
Tannins	+
Flavonoids	+
Phenols	++
Anthranoids	+

Key: + = Present; **++** = Strongly present; **-** = Absent

Table 2: Zones of Inhibition of Ethanolic Extract of *Garcinia kola* Seed on Some Pathogenic Microorganisms

Conc. (mg/ml)	Zones of Inhibition /Organisms					
	S.typhi	P.aeruginosa	E. coli	A. flavus	A.niger	C.albican
20 (Control)	38	59	54	26	26	28
100	18.00	14.50	25.00	4.00	4.00	4.00
200	22.50	16.00	29.00	4.00	4.00	4.00
300	28.00	22.00	35.00	4.00	4.00	4.00

Values of mean ± Standard error of mean (SEM) of duplicate experiment. Value > 4 indicates activity.

Table 3: Minimum Inhibitory Concentration (MIC) of Ethanol Extract of *Garcinia kola* Seed on Some Pathogenic Microorganisms

Conc (mg/ml)	MIC/Test Organisms					
	S.typhi	P.aeruginosa	E. coli	A. flavus	A.niger	C.albican
0 (Control)	+	+	+	+	+	+
40	+	+	+	+	+	+
8	+	+	+	+	+	+
1.6	+	+	+	+	+	+
0.32	+	+	+	+	+	+
0.064	-	-	-	+	+	+
0.0128	-	-	-	+	+	+
0.00256	-	-	-	+	+	+

Key: + = Turbidity; - = No Turbidity

Discussion

The results obtained from this study showed that the ethanol extract of *Garcinia kola* seed at different concentrations has some degree of inhibitory activities against the bacterial isolates used, with more inhibitory effect on *E. coli* at a concentration of 300mg/ml. However, the extract has no inhibitory effect on the fungal isolates. From table 2, there is an increase in the zones of inhibitions with increase in the concentration of the extract against the test bacteria, the zones of inhibition are in the range of 14.50mm-35mm. Table 3 shows the minimum inhibitory effect of extract against the test organisms, with E. coli been the most sensitive at a concentration of 0.0128mg/ml compared to the other test organisms. The antimicrobial activity of the extract against the test organisms, is due to the presence of bioactive components such as saponins, tannins, and phenolic compounds as shown in this study. Flavonoids have been reported to exhibit anti-inflammatory, anti-allergic effects, analgesic and antioxidant properties (Hodek *et al*., 2002). The presence of Cardiac glycoside in *Garcinia kola* extract has been attributed to its therapeutic value in the treatment of cardiac infections along with other ailments such as cough, and chest pain among Yoruba tribe of southwestern Nigeria.

The presence of Tannins in the extracts may be effective for the treatment of intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003), and may as well explain the strong inhibitory effect of the extract on *E. coli*. The antimicrobial effect of the extract in this study, agrees with the work of Okigbo and Mmeka, (2008) who reported that aqueous and ethanolic extract of *Garcinia kola* have inhibitory effect against *E. coli* but no effect on *Candida albicans.* The insensitivity of the fungal strains to the extract can be attributed to the antimicrobial resistance seen among some species of micro-organisms, mainly as a result of antimicrobial resistance genes that they may possess. Since the plant produced good inhibition zones against the bacterial isolates, particularly *E. coli*, it is expected that the seed could be used to treat infections and diseases caused by these organisms, and if the active ingredients are isolated and possibly crystallized, therapeutic antibiotics could be produced from the plant.

Conclusion

The ethanolic extract of *Garcinia kola* (seed) was effective against the bacterial isolates, therefore, holds a great promising potential as antibacterial agents if well exploited, especially in the face of increasing challenges of antibiotic resistance in micro-organisms.

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