Antibacterial and Antioxidant Screening of Ethanol Extract of *Garcinia kola* Seed

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Abstract
Garcinia kola is a medicinal plant that has been used for centuries in the treatment of ailment in sub-Saharan Africa, this is because all parts of this plant have been mentioned to be very important but the seed is the most used part. This study was conducted to test for the antimicrobial and antioxidant activity of ethanol extract of G. kola seed against some microorganisms such as Streptococcus pyogenes, Salmonella paratyphi, Staphylococcus aureus and Escherichia coli. Agar well diffusion method was used to determine the inhibitory effect of different concentration of the extract on the test organisms. The extract was found to inhibit the growth of these organisms as it gave wider zones of inhibition in Streptococcus pyogenes, 17 ± 1.0 mm, and Staphylococcus aureus, 8.0 ± 0.0 mm, and minimal inhibitory zones in Escherichia coli, 6.0 ± 0.0 mm, and Salmonella paratyphi, resistant. Garcinia kola seed showed a slightly lower percentage inhibition when compared to that of vitamin C in antioxidant screening as it gave percentage inhibition of 57.11 for 0.5 mg/ml, 57.32 at 0.25 mg/ml, 53.77 at 0.125 mg/ml, 41.53 at 0.0625 mg/ml and 53.77 at 0.03125 mg/ml. As a result G. kola exhibit antioxidant properties towards preventing free radical from causing damage to the cell thus, this provides justification for its medical use in the treatment of different diseases.

Keywords: *Antibacterial, antioxidant, bitter kola, DPPH, inhibition, vitamin C*

Introduction

The medicinal values of plants lies in the chemical substances present in the parts of the plant such as seed, leaves bark and root. These substances produce definite physiological action in the human body. Okwu, (2003) reported that plants are pools of potential antimicrobial compounds for pharmaceutical need. The array of active compounds derived from them have impressive pharmaceutical properties such as analgesics, aesthetic, antibiotics, because, medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical inter-mediates and chemical entities for synthetic drugs, due to the presence of secondary metabolites which accumulate in the various parts of these plants conferring on them their pharmacological relevance (Ncube *et al.*, 2008). The Seeds, herbs, vegetables, bark, and roots accumulate in their cells a great variety of phytochemical compounds such as alkaloids, tannins, saponins, phenolic compounds (Okwu, 2005).

Garcinia kola otherwise known as bitter cola has been long used for centuries in sub-Saharan Africa for the treatment of various ailments and research has taken a look and found out why it is effective (Uko *et al.*, 2001). *Garcinia kola* is a fruit- bearing plant which belongs to the

family Clusiaceace or Guttiferae. In Nigeria it is called namijingoro in Hausa, orogbo in Yoruba and aki-iluin Igbo. It is found mainly in the tropical forest region of Central and West Africa (Uko *et al.,* 2001). It is predominant in the rainforest belt of southern Nigeria (Agada and Braide, 2009). *Garcina kola* is one of such plants that have been frequently used for its nutritional values as well as for its medicinal virtues, all parts of this plant including the seed, leaf, stem, bark and root have been mentioned in many ethnobotanical and pharmacological studies, although the seed remains the most used part (Okwu, 2003).

In addition to the antimicrobial properties of *Garcinia kola*, there are also antioxidants present which work by neutralizing the action of toxic or harmful chemicals such as free radicals present in food or in the body or by inhibiting oxidation. Antioxidants are known to terminate chain reactions in lipid peroxidation, by removing free radical intermediates and inhibit other oxidation reactions. The body's internal production of antioxidants is not sufficient to neutralize all the free radicals, hence there is need for supplementary dietary intake of antioxidants to maintain health and prevent diseases associated with free radicals (Oloyede and Afolabi, 2012).

Despite modern development in the treatment of diseases, herbal remedies have been continuous and universal. Modern medicines have always depended on herbal extracts from plants as fundamental source of therapeutic ingredients. Some naturally occurring substances in plants play significant role in plant disease resistance and thus most bacteria are sensitive to extract from these plants. This research aims on the proper scrutiny of ethanolic extract of *Garcinia kola* seed with interest channelled towards the effect of its antibacterial and antioxidant nature.

Materials and Methods

Materials

Collection of Plant Material

The seeds were purchased from Garki main market in FCT, Abuja. The seed coat was removed to obtain the seed. The seeds were chopped into smaller pieces and dried at room temperature until a specific weight was obtained over a period of time. The seed were pulverized using a mortar and pestle and it was stored in polythene bags and placed at room temperature until when used.

Isolates

The clinical isolates used were *Staphylococcus aureus, Streptococcus pyogenes, Salmonella paratyphi* and *Esherichia coli* isolated from patients at University of Abuja Teaching Hospital, Gwagwalada, FCT, Nigeria.

Chemicals and Reagents

The chemicals and reagents used include; Ethanol, Ferric chloride, Hydrochloric acid, Chloroform, Conc. H_2SO_4, Sodium hydroxide, Ammonia, Acetic anhydride, Dimethyl sulphur oxide (DMSO), Methanol, Glacial acetic acid, 2, 2- diphonyl-1-picrylhydrozyl (DPPH), Ascorbic acid, Muller Hinton agar, Nutrient broth, Nutrient agar, Mayer' s Reagent, Olive oil.

Methods

Preparation of Ethanol Extracts

This was done according to the methods of Falope *et* al. (1993). One hundred grams (100 g) of the powdered sample was weighed into a clean dried extraction bottle and 500 ml of

ethanol was added. The mixture was vigorously shaken and left to stand for 72 hours with occasional shaking to allow extraction at room temperature. After 72 hours, the mixture was filtered using a Whatman filter paper (No. 1). The resulting filtrate was concentrated in a water bath where it was evaporated to give the required brownish-black residue. The extract was stored in air tight sample bottles in a refrigerator until required for use.

Concentration of the Extract

This was done according to the methods of Lawal *et al.* (2011). Five hundred grams of the extract was dissolved in 1 ml of dimethyl sulphoxide (DMSO) to obtain 500 g/ml, other concentrations were then prepared using two-fold doubling dilution, thus, reducing the stock concentration from 500 g/ml to 250 g/ml, 125g/ml and 62.5 g/ml respectively.

Phytochemical Analysis

The extract was analysed according to methods of Harbone (1998); Joshi *et al.* (2013) and Kokate (2001) for the presence of tannins, saponins, flavonoids, cardiac glycosides, alkaloids, terpenoids, steroids and anthraquinones.

Antimicrobial Activity

Sensitivity Test

The antimicrobial activity of the extract was determined using the Agar well diffusion method as described by Ogundipe *et al.* (2000). For this method, four perforations were performed using a 6 mm cork borer under aseptic conditions on Mueller Hinton agar Petri dish previously seeded with the appropriate bacterial culture (adjusted to 0.5 McFarland standard). About twenty five microliter (25 µL) each of different concentration of the extract solution was aseptically lodged in the hole. These dishes were kept for 15-30 minutes at room temperature to allow the extract diffuse properly and dry to a considerable level before incubation at 37°C for 24 hours. After the incubation period, the dishes were examined for inhibitory zones; the antibacterial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. Chloramphenicol (30 µg) was used as control. The experiment was done in triplicates.

Minimum Inhibitory Concentration

To determine the minimum inhibitory concentration (MIC) of the crude extract according to the methods of Vollekova *et al.* (2001) and modified by Usman *et al.* (2007), equal volumes of nutrient broth seeded with the test organisms was aseptically introduced into sterile test-tubes, different concentration of the extract was introduced into the same test tubes. The tubes were incubated for 24 hours at 37 ^{0}C, the MIC of each was determined by measuring the optical density using a spectrophotometer and comparing the result with those with and without the inoculum.

Minimum Bactericidal Concentration

For minimum bactericidal concentration, it was analysed using the method described by Andrews (2001). The tube that indicates no growth when inoculated in freshly prepared nutrient agar and incubated for another 24 hours and shows no growth after incubation is considered the minimum bactericidal concentration.

Antioxidant Activity

The free-radical scavenging activity was evaluated by accessing its discolouration of 2, 2-diphonyl-1-picrylhydrozyl radical (DPPH) in methanol by a slightly modified method of Brand-Williams *et al.* (1995).The following concentrations of the extract was tested (0.05,

0.1, 0.5, 1.0, 2.0 and 5.0 mg/ml). The decrease in absorbance was monitored at 517nm. Vitamin C was used as the antioxidant standard at concentrations (0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 mg/ml). One millilitre (1 ml) of the extract was placed in a test tube and 3 ml of ethanol was added followed by 0.5 ml of 1mM of DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A - B}{A} \times 100$$

Where A = absorption of the blank sample without extract.

B= the absorption of the extract.

Results

The percentage yield and macroscopic characteristics of the crude extracts of the *Garcinia kola* seed are presented in Table 1.The phytochemical constituents of the ethanol extract of *Garcinia kola* is indicated in Table 2. Alkaloids, flavonoids, terpenoids, saponins, tannins, and steroids, glycosides were observed to be present in the extract while anthraquinones and carbohydrate were absent in the extract.

The antibacterial activity of *Garcinia kola* seed ethanol extract is indicated in Table 3. *Streptococcus pyogenes* was more susceptible to the extract while *Escherichia coli* was the least susceptible to the extract. The antibacterial activity against the test organism *S. pyogenes, S. paratyphi* and *S. aureus* was concentration dependent ($p<0.05$). Table 4 shows the minimum inhibitory concentration (MIC) of the ethanol extract against the test bacteria. The MIC of the extract against *Streptococcus pyogenes*was 31.3 mg/ml, 250 mg/ml against *Salmonella paratyphi* and 62.5 mg/ml against *Staphylococcus aureus*. The MIC of the extract against *E. coli* was 125 mg/ml.

Table 5 shows the minimum bactericidal concentration of ethanol extract of *G. kola* seed against test bacteria. The extract was observed to be bactericidal on *Streptococcus pyogenes* at 125 mg/ml for *Staphylococcus aureus* the MBC was at 500mg/ml. The MBC of the extract against *E. coli* was 250 mg/ml. Table 6 shows the antioxidant assay of ethanol extract of *Garcinia kola* seed. The seed extract exhibited potent DPPH scavenging activity.

Table 1: Yield of Ethanol Extract of *Garcinia kola* Seed

Extract	Colour/texture	Yield (%)
Ethanol extract	dark brown/ gel	11

Table 2: Phytochemical constituents of ethanolic extract of *G. kola* seed

S/N	Phytochemical Components	Ethanol extract
1.	Alkaloids	+
2.	Anthraquinones	-
3.	Flavonoids	+
4.	Saponins	+
5.	Tannins	+
6.	Terpenoids	+
7.	Steroids	+
8.	Carbohydrate	-
9.	glycosides	+

(+) present; (-) absent

Table 3: The Zones of Inhibition of Ethanolic Extract of *G.Kola* on Tested Organisms

Test organisms	Conc.	Ethanolic extract	Chl (30µg)
Streptococcus pyogenes	500 mg/ml	17.0 ± 1.0^{a}	21.0 ± 0.0
	250 mg/ml	12.0 ± 1.0^{b}	
	125 mg/ml	10.0 ± 0.1^{b}	
	62.5 mg/ml	6.0 ± 0.1^{c}	
Salmonella paratyphi	500 mg/ml	NI	19.0 ± 0.0
	250 mg/ml	NI	
	125 mg/ml	NI	
	62.5 mg/ml	NI	
Staphylococcus aureus	500 mg/ml	8.0 ± 0.0^{a}	16.0 ± 0.0
	250 mg/ml	6.0 ± 0.0^{b}	
	125 mg/ml	NI	
	62.5 mg/ml	NI	
Escherichia coli	500 mg/ml	6.0 ± 0.0^{a}	22.0 ± 0.0
	250 mg/ml	NI	
	125 mg/ml	NI	
	62.5 mg/ml	NI	

Values are Zone diameter if inhibition in mm ± Standard Error; NI = No Inhibition
Zones with different alphabets are significantly different ($p<0.05$)

Table 4: Minimum Inhibitory Concentration of Ethanolic Extract

Test organisms	Ethanol ext. (mg/ml)						
	500	250	125	62.5	31.3	15.6	7.8
Streptococcus pyogenes	-	-	-	-	-	+	+
Salmonella paratyphi	-	-	+	+	+	+	+
Staphylococcus aureus	-	-	-	-	+	+	+
Escherichia coli	-	-	-	+	+	+	+

(-): turbidity less than growth control
(+): turbidity higher than growth control

Table 5: Minimum Bactericidal Concentration of Ethanolic Extract

Test organisms	Ethanol ext. (mg/ml)						
	500	250	125	62.5	31.3	15.6	7.8
Streptococcus pyogenes	-	-	-	+	+	+	+
Salmonella paratyphi	-	-	+	+	+	+	+
Staphylococcus aureus	-	+	+	+	+	+	+
Escherichia coli	-	-	+	+	+	+	+

Keys: + Growth- No growth

Table 6: Antioxidant assay of *Garcinia kola* seed extract

Concentration (mg/ml)	Percentage inhibition (%)	
	Control (Vitamin C)	Ethanol extract
0.5	79.03	57.11
0.25	76.82	57.32
0.125	79.40	53.77
0.0625	79.07	41.53
0.03125	81.24	53.77

Values are percentage Inhibition of DPPH

Discussion

The phytochemical analysis of ethanol extract of *G. Kola* seed in this research showed the presence of tannins, alkaloids, steroids, glycosides, saponins and flavonoid. This is in agreement with report of Omwirhiren *et al*. (2017) who reported the presence of alkaloids, saponins, tannins, flavonoids, glycosides, steroids, saponins and cardiac glycosides. Many higher plants are known to possess antibacterial agents and indeed extracts of plants from different parts of the world have been known to produce antimicrobial properties as observed

in this work. The antibacterial activity of the extracts can be attributed to the synergistic action of some bio reactive substances such as the alkaloids, tannins, saponins and flavonoids among others in the extracts (Matasyoh *et al.*, 2009). Ezeigbo *et al*. (2016) reported potent antibacterial effect of extracts of *Garcinia kola* on *Salmonella* species, *Escherechia coli*, *Shigella* species and *Pseudomonas* species; their study revealed that the methanol extract yielded the highest zones of inhibition ranging from 23.5-30.8 mm this was followed by ethanol extract ranging from 17.1-24.5 mm while aqueous extract had the least inhibition from 13.1-21.2 mm. However, the ethanol extract results obtained from this work yielded zones of inhibition ranging from 6.0 – 17.0 mm for *Streptococcus pyogenes, Salmonella paratyphi* was resistant, 0.0-8.0 mm for *Staphylococcus aureus* and 0.0 – 6.0 mm for *Escherichia coli* with each zone increasing with increase in concentration, that is, the higher the concentration of extract, the wider the zones of inhibition obtained depending on the organism and their susceptibility to the extract, the results indicate that *Streptococcus pyogenes* was more susceptible followed by *Staphylococcus aureus, Escherichia coli,* with the least susceptible been *Salmonella paratyphi.* However, there is no significant difference ($p \leq 0.05$) on the effect of *G. kola* seed extract on the test organisms.

The broad spectrum of activity exhibited by the alcoholic extract of *Garcinia kola* against Gram positive and Gram negative bacteria in this study (Table 3) explains their use in a wide range of ailments in Nigeria and this maybe as a result of the bioactive components present (Table 2) which is in line with that of Ogunmoyole *et al*. (2012) who reported the presence of phenolic acids, flavonoids and vitamin C in extracts of *G. kola*.

The ethanolic extract of *G. kola* seed also exhibited minimum inhibitory concentration on the test organisms (Table 4) as the MIC value for *S. Pyogenes* was 31.3 mg/ml, 250 mg/ml for *Salmonella paratyphi*, for *S. aureus,* it was at 62.5 mg/ml and for *Escherichia coli* the MIC was at 125 mg/ml. Ethanolic extract of *G. kola* seed was bactericidal on the test organisms as it exhibited MBC at 125 mg/ml for *Streptococcus pyogenes,* 250 mg/ml for *Salmonella paratyphi.* For *Staphylococcus aureus,* the MBC was 500 mg/ml while *E. coli* was 250 mg/ml (Table 5).

The seed extract exhibited potent DPPH scavenging activity (Table 6). Radical scavenging activity is very crucial to the survival of living organisms, due to the deleterious role of free radicals in foods and biological systems. Because 1, 1 diphenyl-2-Picrylhydrazyl (DPPH) radicals are very stable but can be easily scavenged by antioxidants, they have been used to evaluate the free radical scavenging activity of natural compounds (Jain *et al*., 2017). DPPH turns to a stable diamagnetic molecule when protonated (Ogunmoyole *et al*., 2012). From the result obtained from this research, it indicates that the ethanol extract shows a high antioxidant percentage inhibition which is different from that of the control (vitamin C) as a result, there is significant difference ($p \geq 0.05$) between the ethanol extract and vitamin C (control) but there is no significant difference between the concentrations ($p \leq 0.05$). This result is similar to the report of Ogunmoyole *et al*. (2012), comparing the antioxidant activity of ethanol and aqueous extract of *G. kola* reported that the ethanol extract showed a significantly higher radical scavenging effect than the aqueous extract.

Studies have shown that antibiotic resistance occurs as a result of intrinsic mechanisms that prevent bacteria from destruction (McDonnell *et al*., 2001). The management of resistant bacteria therefore, is an attractive strategy using medicinal plants which cater for about 80% of the vast populace that rely mostly on herbs for their medicines, (Maiyo *et al*., 2010; Temitope *et al*., 2016; Neethu *et al*., 2016).

Conclusion

In conclusion, the present study showed that *Garcinia kola* contains an array of secondary metabolites such as alkaloids, tannins, flavonoids, saponins, steroids, glycosides and terpenoids and these metabolites constitute to its antibacterial effects against Gram positive and Gram negative bacteria. The antibacterial effect was mostly bacteriostatic. The seed of *G. kola* is a potent antioxidant with percentage inhibition values slightly less than that of vitamin C which is known for its high antioxidant nature. The phytochemical constituents, potent antibacterial effect and valuable antioxidant potential of the extract of *Garcinia kola* seed suggest that it could have unique applications alternative medicine in developing countries.

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