Phytochemical and Antibacterial Properties of *Garcinia kola Seeds* (Bitter kola) on *Escherichia coli* and *Staphylococcus aureus*

Valentine N. Unegbu[1]
Florence N. Okey-Ndeche[2]
Charity N. Obum-Nnadi[2]
Pius I. Egwuatu[3]

[1]Department of Microbiology, Spiritan University Nneochi, Abia State, Nigeria
[2]Department of Microbiology, Veritas University, Abuja, Nigeria
[3]Department of Microbiology, Renaissance University, Ugbawka, Enugu State, Nigeria

Abstract. The different parts of plants such as seed, leaves, barks and root contain medicinal substances. These substances produce definite physiological action in the human body. This present study was aimed to investigate the phytochemical and antibacterial properties of methanolic, ethanolic and aqueous extracts of *Garcinia kola* (bitter kola) on *Staphylococcus aureus* and *Escherichia coli*. The phytochemical constituents were carried out using standard methods. The antibacterial activities of the plant extracts was determined using agar-well diffusion method. The Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant extracts on the test isolates were determined by micro-broth dilution method. Phytochemical analysis shows that Phenols, flavonoids, glycosides, tannin, saponin, alkaloids and anthraquinolones were present in varying concentrations of the different extracts. The methanol extract of *G. kola seed* possesses more antimicrobial activity (17-25 mm) in a concentration dependent manner than the ethanol extract (10-21 mm) and then aqueous extract (7-17mm). The MIC of different extracts of *S. aureus* was between 12.5mg/ml to 50mg/ml while that of *E. coli* was also between 12.5mg/ml to 50mg/ml. The MBC of different extracts of *S. aureus* and *E. coli* isolates were both between the ranges of 25 to 100mg/ml. It can be concluded that some secondary metabolites present in *Garcinia kola seed* was responsible for the inhibition of the bacteria observed in this study; thus, the test plant could be used to manufacture drugs that could be used to treat infections caused by the test organisms.

Key words: Garcinia kola, Ethanolic, Aqueous, Methanolic, Staphylococcus aureus, Escherichia coli.

Introduction

Garcinia kola, mostly known in Nigeria as bitter kola is found in moist forest and grows as a medium size tree, up to a height between 10 to 12 m high (Mboto et al., 2009: 557-559). It is a dicotyledonous plant belonging to the family of plant called Guttiferae. It is cultivated and distributed throughout west (such as Nigeria and Sierra Leone) and central Africa. It is commonly called “Agbilu” in Igbo land and “Namijin goro” in Hausa and “Orogbo” in Yoruba land of Nigeria (Mboto et al., 2009: 557-559). The edible seed is used as a substitute for true kola nut (*Cola nitidais*) in most Nigerian homes (Mboto et al., 2009: 557-559). The antimicrobial substances in the seed and the mechanical cleansing effects are seen as major beneficial effects of chewing this nut (Nwaokorie et al., 2010: 509-514).

Phytochemical analysis of extracts from different part of the plant show that they contain reasonable amounts of phenolic compounds like xanthones, biflavonoids (GB-

1,GB-2) and benzophenones (Adegboye et al., 2008: 3934-3938; Jayalakshmi et al., 2011: 124-128).

Their antibacterial activities are due to the presence of some phytochemical substances like saponins, phenols and flavonoids especially biflavonoid type GB1 which are well known for their antioxidant activities (Adegboye et al., 2008: 3934-3938).

Scientific evaluations revealed that the leaves, seeds, stem and bark are used to treat some illness like vomiting, liver disorders, fever, cold and cough etc (Sibanda and Okoh, 2008: 149-154). The seeds are also used to treat throat, stomach and respiratory disorders; and bronchitis. The seed extract is also effective against some gram positive and negative bacteria like *S. aureus* and *E. coli* respectively (Gill, 1992: 276).

The type of solvents used has an effect on the nature of compounds extracted and the resulting bioactivity of the extract (Doughari et al., 2008: 007-013; Eloff, 2005: 1161-1166). This clearly implies that polarity of solvents (non-polar, polar and less polar) play a vital role in the extraction of bioactive compounds, which influence the antimicrobial activity (Basri and Fan, 2005: 26-29; Parekh, 2006: 832-836). It is important for the efficiency of extraction to be optimized in order to ensure that many potential active constituents are extracted as possible.

Presently there are global problems of antibiotic resistance to infections coupled with the emergence of new and re-emerging diseases. There is also a belief that the use of plants for medicinal purposes has been associated with less side effects (Odebunmi, 2009: 308-310). There is therefore a need to search for more efficacious and cost-effective antimicrobial agents of natural origin to complement the existing synthetic antimicrobial drugs that are becoming less potent against pathogenic microorganisms (Odebunmi, 2009: 308-310).

The present study therefore looks to investigate the phytochemical and antibacterial properties of different seed extracts of *Garcinia kola* (Bitter kola) on clinical isolates *of Staphylococcus aureus* and *Escherichia coli.*

Materials and Methods

Sample collection

The seeds of *G. kola* were purchased from Eke Agbani market in Enugu State, Nigeria and was authenticated by a Botanist at Nnamdi Azikiwe University Herbarium with a voucher number of NAUH 262B**.**

The preparation was done at the Microbiology laboratory of Spiritan University Nneochi, Abia State. The seeds of *Garcinia kola* were de-husked from the seed coat, cut into bits and placed in a washed tray to dry. The seeds were dried under the sun and with the help of a pestle and mortar, it was crushed to coarse powder. Different containers were used to label and store the powdered form of the seeds.

Test organisms

Bacterial cultures of *Escherichia coli and Staphylococcus aureus* obtained from the laboratory section of the Department of Microbiology, Nnamdi Azikiwe University, Anambra State, Nigeria; were used as antimicrobial test organisms. Their identity was confirmed using cultural, morphological and biochemical test as previously described (Akinnibosun, 2009: 33-37; Okigbo and Mmeka, 2008: 226-229). The bacterial isolates were maintained on nutrient agar slants at 4°C for future use.

Biochemical Identification of the Test Organism

Escherichia coli

The *E. coli* was placed on Eosine Methylene Blue (EMB) agar for 24 hours. A positive result for *E. coli* was indicated with colonies with green metallic sheen. The

distinct colonies with metallic green sheen on EMB agar were pick and confirmed by streaking onto Chromagar *E. coli* medium (Oxoid, Basingstoke, UK). Colonies with a blue/violet appearance were selected and analyzed further by gram staining and biochemical tests (Cheesbrough, 2002: 63-70).

Staphylococcus aureus

Staphylococcus aureus which showed positive result for catalase test was sub-cultured on blood agar and incubated at 37°C for 24 hours. Then, the single colonies were placed on Mannitol Salt Agar (MSA) for 24 hours. A positive result was indicated by smooth circular colonies with yellow color (Cheesbrough, 2002: 63-70).

Standardization of the Tests Organism

The test organisms (*E. coli and S. aureus*) were standardized by the use of 24 hours old broth cultures prepared by inoculating the test organism into 5 ml of nutrient broth and the culture was incubated for 2 hours. The growth of the organism was indicated by the turbid change in color of the nutrient broth, which was adjusted to match the color of the 0.5 McFarland turbidity equivalent standard (Cheesbrough, 2002: 63-70).

Preparation of Aqueous, Ethanolic and methanolic extracts

The extracts were prepared according to the method described by (Akinnibosun, 2009: 33-37).

Preparation of Aqueous extract

Ten grams of dried grinded seed powder was dissolved in 100 ml of distilled water for 24 hours. The mixture was filtered using Whatman's filter paper No. 1 to obtain solution free of solids. The filtrate was dried to concentration at 37°C and stored at 4°C.

Preparation of ethanolic extract

Ten grams of dried grinded seed powder was dissolved in 100 ml of 95% ethanol for 24 hours. The mixture was filtered using Whatman's filter paper No. 1 to obtain solution free of solids. The filtrate was placed into evaporator to drive-off the solvent and stored at 4°C.

Preparation of Methanolic Extract

Ten grams of dried grinded seed powder was dissolved in 100 ml of 95% methanol for 24 hours. The mixture was filtered using Whatman's filter paper No. 1 to obtain solution free of solids. The filtrate was placed into evaporator to drive-off the solvent and stored at 4°C.

Extract Dilution

After preparation of the extract as described, the aqueous, methanolic and the ethanolic extract were reconstituted using sterile distilled H_20 to obtain concentrations of 200, 100, 50, 12.5, 6.25 and 3.13 mg/ml.

Sterility test of leaf extract

The dried grinded seed extracts (ethanol and aqueous) was tested for growth of contaminants according to the methods of (Cheesbrough, 2002: 63-70). Standard dried grounded seed extract (1 ml) was inoculated aseptically onto Nutrient Agar and incubated at 37^{o}C for 24hrs. The plates were observed for sign of growth. No growth on the plates signified sterility of the extracts.

Phytochemical screening of G. kola seed extracts

The sample was screened for the following compounds, alkaloids, saponins, tannins, flavonoids, glycosides, phlobatannin and anthraquinones etc. This was done following standard methods of (Trease and Evans, 1989: 378-386).

Test for Tannins

Two (2) gram of each extract was dissolved in 10ml of distilled water in separate test tubes and 3 drops of 10% ferric chloride ($FeCl_3$) was added to 2ml of the solution.

The occurrence of blackish-blue, green or blackish green coloration indicates the presence of tannins.

Test for phlobatannins

About 0.2g of each extract was boiled with an equal volume of 1% Hcl, the deposition of a red precipitate indicates the presence of phlobatannins.

Test for saponins

About 0.1g of each extract was dissolved in 5ml of distilled water and shaken vigorously. The formation of fronthing bubbles which lasted for 10 minutes indicate the presence of saponin.

Test for alkaloids

About 0.5g of each extract was dissolved in 3 drops of Dragendoffs reagent. An orange precipitate indicates the presence of alkaloid.

Test for flavonoids

About 0.2g of each extract was dissolved in 2ml of sodium hydroxide solution. The occurrence of a yellow solution which disappears on addition of Hcl acid indicates the presence of flavonoids.

Test for glycoside

Half (0.5) g of each extract was dissolved in 3ml of Fehling solution. A brick red precipitate indicates the presence of glycosides.

Test for steroids

Five (5) drops of concentrated H_2S0_4 was added to 0.1g of each extract in test tube, a reddish-brown coloration indicates the presence of steroids.

Terpenoids

Four milligrams (4mg) of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated sulphuric acid solution was added slowly and red violet color was observed for terpenoid.

Anthraquinone

To ten milligrams (10mg) of the dissolved extract, magnesium acetate solution was added. Pink colour developed indicates the presence of anthraquinone and no colour change indicates negative.

Antibacterial Assay

The agar-well diffusion technique was used to carry out the antibacterial assay of the bitter kola seed extracts in comparison with standard antibiotic gentamicin (10 mg/ml) *in-vitro* on the isolates according to the method (NCCLS, 2007). Pure culture of the bacteria was grown on nutrient agar. Three colonies of each organism were pick into the Mueller Hinton broth (Oxoid, England), incubated for 4 hours at 37°C and diluted with sterile saline to a density visually equivalent to MacFarland standard. Using a sterile 5 mm diameter cork borer, four wells were cut in the agar to which the two extracts of *Garcinia kola seed* were added, as well as the standard drug, gentamicin (GEN, 10 mg/ml) and sterile water separately, which served as the positive and negative controls respectively. The plates were placed on the bench for 30 minutes for pre-diffusion of the extract to occur. The plates were incubated at 37°C for 48 hours. The zones of inhibition were measured with the use of a metric rule.

Determination of Minimum Inhibitory Concentration (MIC)

The micro broth dilution method was employed in the determination of the Minimum inhibitory concentration (MIC) of *Garcinia kola seed* extracts against the test organisms. To each 5ml of the various extracts in different test tubes was added 5ml of nutrient broth each and serially diluted out to various concentrations ranging from 200 to 3.13mg/ml. A loop full of each test organisms was inoculated into each of the test tubes and incubated

at 37ºC for 24 hours. The MIC was the lowest concentration of the seed extracts that inhibited growth (Cheesbrough, 2002: 63-70).

Determination of Minimum Bactericidal Concentration (MBC)

Briefly, 1ml bacterial culture was pipetted from the mixture obtained in the determination of MIC tubes which did not show any growth and were sub-cultured onto nutrient agar and incubated at 37ºC for 24hours.This was obtained by streaking out the samples from the MIC tubes that showed no visible growth on nutrient agar plates. The lowest concentration of the sample that showed no growth was noted and recorded as the minimum bactericidal concentration (Cheesbrough, 2002: 63-70).

Results

Table 1 shows the Phytochemical components of methanolic, ethanolic and aqueous extracts of *Garcinia kola*. Steroids and Phlobatanin were absent in all extracts of *Garcinia kola* while Phenols, flavonoids, glycosides, tannin, saponin, alkaloids and anthraquinolones were present in varying concentrations of the extracts.

Table 1. Phytochemical composition of *Garcinia kola* (Bitter kola)

Phytochemical components	ME	EE	AE
Phenol	+++	++	+
Flavonoids	+++	++	+
Steroids	-	-	-
Glycosides	+	+	+
Tanins	++	+	+
Saponins	+++	++	+
Alkaloids	+	+	+
Anthraquinolones	+	+	+
Phlobatanin	-	-	-
Terpenoids	+	+	+
KEY: - = Absence + = Slightly present + = Moderately present ++ = Highly present			

The antibacterial activities of aqueous, ethanolic and methanolic seed extracts of *Garcinia Kola on S. aureus and E. coli* is found on Table 2. The mean zone diameter of inhibition for *S. aureus* on the different extracts was between the range of 7mm to 24mm while that of *E. coli* was between 7mm to 25mm.

Table 2. Antibacterial activities of aqueous, methanol and ethanol seed extract of *G. kola seeds* on *S. aureus* and *E. coli*

Isolates	Mean zone diameter of inhibition (mm)						Extracts
S. aureus	16	12	9	7	15	0	AE
S. aureus	24	22	19	7	15	0	ME
S. aureus	19	16	12	10	15	0	EE
E. coli	17	15	11	7	17	0	AE
E. coli	25	24	22	20	17	0	ME
E. coli	21	19	17	15	17	0	EE
Key:							

AE = Aqueous Extract
ME = Methanolic Extract
EE = Ethanolic Extract
+C = Positive control (gentamicin, 10mg/ml)
-C = Negative control (sterile water)

The MIC and MBC of seed extracts of *G. kola on S. aureus and E. coli* is found on Table 3. The MIC and MBC of aqueous seed extract on *S. aureus* are 50 and 100mg/ml respectively while the MIC and MBC of methanol seed extract on *S. aureus* are 12.5 and 25mg/ml respectively. The MIC and MBC of ethanol seed extract on *S. aureus* are 25 and 50mg/ml respectively. The MIC and MBC of the extracts on *E. coli* are found on Table 3.

Table 3. Minimum Inhibitory and Bactericidal Concentration (MIC and MBC) of seed extracts of *G. kola on S. aureus and E. coli*

Isolates	Concentration of Extracts (mg/ml) MIC (mg/ml)	MBC (mg/ml)	Extracts
S. aureus	50	100	AE
S. aureus	12.5	25	ME
S. aureus	25	50	EE
E. coli	50	25	AE
E. coli	12.5	25	ME
E. coli	25	100	EE

Key:
AE = Aqueous Extract
EE = Ethanol Extract
ME= Methanol Extract

Discussion

Data's from the phytochemical analysis revealed the presence of phenols, saponins and flavonoids in high amount in methanolic extract and moderate amount in ethanolic extract respectively. Alkaloids, glycosides, anthraquinones and terpenoids were found in slight amount in all extracts of *Garcinia kola seeds.* This was corroborated by the works of (Adegboye et al., 2008: 3934-3938; Eminedoki et al., 2010: 232-237) who also found similar products in *G. kola*. Adesuyi et al. posited that 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, in addition to superoxide anion radicals, leading to the promotion of good health (Adesuyi et al., 2012: 9-14). Flavonoids is also associated with antioxidant properties, analgesic and anti-inflammatory properties, which support the usefulness of *G. kola* in the treatment of various infections caused by gram positive and gram-negative organisms (Adesuyi et al., 2012: 9-14).

Results from the solvent extracts used showed that methanol, followed by ethanol extracted the active compounds better compared to aqueous solvent. This could be attributed to the high volatile property of methanol. Similar results were found in the works of (Adegboye et al., 2008: 3934-3938; Nosiri and Abba, 2010: 2-6).

The synergistic action of some isolated bio-reactive substances such as the phenols, glycosides, alkaloids, tannins, saponins, flavonoids, anthraquinolones and terpenoid can be attributed to the antimicrobial activity observed in this study (Jonathan and Fasid, 2005: 83-87). Antimicrobial activities are features associated mainly with higher plants which has contributed to the production of alternative drugs to cure many

bacterial infections hitherto resistant to many conventional antibiotics (Jonathan and Fasid, 2005: 83-87).

It was also shown that the methanolic and ethanolic extracts of *Garcinia kola* inhibited the growth of both the *E. coli and S. aureus* than the aqueous extract in a concentration dependent manner. Although extract produced with methanol exhibited more inhibitory effect when compared with ethanol, this could be because of high active compounds present in the extract (Ezeifeka et al., 2004: 49-154). The variation in the antibacterial activities is presumed to be due to difference in the quantity of compounds present in those plant extracts (Ezeifeka et al., 2004: 49-154).

More so, result from this work shows a greater zone of inhibition produced by the methanolic extracts of *G. kola* at all concentrations used compared to that produced by the positive control drug gentamicin. This indicates the possibility that *G. kola* could serve as a better and alternative drug to treat infections caused by *S. aureus* and *E. coli* compared to most conventional antibiotics used presently in the world. Similar result was discovered in the work of (Njume et al., 2011: 822-827).

The results of the Minimum Inhibitory Concentration and minimum bactericidal concentration of the seed extract against the test organisms showed that the extracts were effective when compared with the zones of inhibition of the standard antibiotics. This agrees with the work (Esimone, 2007: 232-237) that reported *G. kola* seed extracts exhibited bactericidal effects very well when compared to standard antibiotics.

Conclusion

Phytochemical analysis shows that Phenols, flavonoids, glycosides, tannin, saponin, alkaloids and anthraquinolones were present in varying concentrations of the different extracts. Methanolic, ethanolic and aqueous extract of *Garcinia kola* seed possesses antimicrobial activity in a concentration dependent manner. This is attributed to the presence of these phytochemical compounds like Phenols, flavonoids, glycosides, tannin, saponin, alkaloids and anthraquinolones identified in this study.

It can also be concluded that the methanol extract of *G. kola seed* possesses more antimicrobial activity (25–17mm) in a concentration dependent manner than the ethanol extract (10–21mm) and then aqueous extract (7–17mm).

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