



Antibacterial Activities of Bitter Kola on *Streptococcus pyogenes* and *Staphylococcus aureus*

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ABSTRACT

Bitter kola (*Garcinia kola*) is known mostly for its antimicrobial potentials. Resistance to drugs especially antibiotics has become a major challenge facing the medical fraternity today. These challenges call for renewed strategies on treatment, especially in the development of new antimicrobials using plant and its derivatives. The study was carried out to test for the antibacterial activity of bitter kola extracts on *Streptococcus pyogenes* and *Staphylococcus aureus* collected from Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. The phytochemical screening performed on bitter kola extracts revealed the presence the followings saponins, alkaloid, flavonoid, glycoside, carbohydrate, anthraquinones, unsaturated steroid, and tannins. The agar-well diffusion method was used to determine the inhibitory effect of bitter kola on the test microorganisms. *Streptococcus pyogenes* was most susceptible to ethanol extract of the bitter kola with an inhibition zone of 12 to 24mm, while *Staphylococcus aureus* was most susceptible to aqueous extract with an inhibition zone of 10-28mm. The aqueous extract exhibited activity against *Streptococcus pyogenes* and *Staphylococcus aureus* with minimum inhibitory concentration (MIC) values of 50mg/ml and 25mg/ml respectively, while the ethanol extract showed activity against *Streptococcus pyogenes* and *Staphylococcus aureus* with MIC values of 25mg/ml and 12.5mg/ml respectively. The extracts also showed bactericidal activity against both organisms. The minimum bactericidal concentration (MBC) value of aqueous extract for *Streptococcus pyogenes* and *Staphylococcus aureus* was 50mg/ml and 25mg/ml respectively, while the MBC value of the ethanol extract for *Streptococcus pyogenes* and *Staphylococcus aureus* was 25mg/ml. The result of this study shows that bitter kola could be better option to some of the antibiotics commonly used to treat infections caused by *Streptococcus pyogenes* and *Staphylococcus aureus*.

Keywords: *Garcinia kola*; antimicrobial; *Streptococcus pyogenes*; *Staphylococcus aureus*

INTRODUCTION

Bitter kola is a species of flowering plant. It is a powerful nut scientifically known as *Garcinia kola* and belongs to the family *Guttiferae*. It is a tropical tree of evergreen forests found in moist semi deciduous forest and savannah regions where the average water availability is equivalent to 2000–2500 mm of rainfall per annum with temperature ranges of 21°C to 32°C and minimum relative humidity of 76.34% (Eyog-Matig *et al.*, 2007). Bitter kola is known as *Orogbo* in Yoruba, *Namijin-goro* in Hausa and *Akuilu* in Igbo.

Garcinia kola has been recognized as an indigenous medicinal plant found in rain forest of central and western Africa, especially

Benin, Cameroon, Democratic Republic of Congo, Cote d'Ivoire, Gabon, Ghana, Liberia, Nigeria, Senegal and Sierra Leone (Eyog-Matig *et al.*, 2007). The seeds of *Garcinia kola* extracts possess many medicinal properties in the forms of anti-inflammatory, antibacterial, antimicrobial, antidiabetic, purgative and antihepatotoxic activity (Akoachere *et al.*, 2002; Esimone *et al.*, 2007).

The medicinal properties of the plant have been a subject of numerous investigations. The seed commonly known as bitter kola are used by communities for the treatment of bronchitis

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and throat infections (Iwu *et al.*, 1999). It is also used to prevent and relieve colic, curing of head or chest colds and relieve cough (Farombi, 2000). The stems and twigs of the plant are used as chewing sticks in maintenance of oral hygiene (Ndukwe *et al.*, 2005). Bitter kola can also be used as remedy for dysentery, osteoarthritis, antidote against poisoning. In recent years, ground seeds have been used as an industrial bittering agent in some Nigerian breweries (Okoro and Aina, 2007). The antimicrobial properties of the *G. kola* seeds are attributed to flavonoids (Anegbeh *et al.*, 2006). Flavonoids exhibit a wide range of biological activities one of which is their ability to scavenge for hydroxyl radicals and superoxide anion radicals.

Staphylococcus aureus is one of the most harmful species of staphylococci encountered. It is the leading cause of bacteremia, pneumonia, myocarditis, acute endocarditis, pericarditis, osteomyelitis, encephalitis, meningitis, mastitis, and scalded skin syndrome (Murray *et al.*, 2003). The emergence of resistance of hospital-acquired *Staphylococcus aureus* to conventional and new generation antibiotics highlights this species as a potential pathogen.

Streptococci are Gram positive, non-motile, facultative anaerobes, spherical bacteria that characteristically form long chains of cocci and occasionally in pairs during growth (Brooks *et al.*, 2013; Willey *et al.*, 2014). It is associated with a wide range of infections including pharyngitis and impetigo to invasive necrotizing fasciitis, streptococcal toxic shock syndrome, puerperal fever, pneumonia, cellulitis, erysipelas, bacteremia/sepsis, streptococcal pyoderma and scarlet fever (Mahon *et al.*, 2015).

The global emergence of multi-drug resistant bacterial strains is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure of infections (Hancock, 2005). Examples of such bacteria include methicillin-resistant *Staphylococcus aureus* (Norrby *et al.*, 2005).

Because of the rate at which bacteria are developing resistance to antibiotics, there is the need for alternative drug before the battle is won over by the microbes (Gibbon *et al.*, 2005). *Staphylococcus aureus* is recognized as one of the major causes of infection in humans

occurring in both the community and the hospital. Therefore the search for new drugs from novel sources such as plant is necessary.

This work is therefore aimed at determining the antibacterial potentials of *Garcinia kola* extract on *Staphylococcus aureus* and *Streptococcus pyogenes* isolated from clinical samples obtained from Ahmadu Bello University Teaching Hospital Shika, Zaria, and Kaduna State, Nigeria.

METHODOLOGY

Collection, Identification and Processing of Plant

Bitter kola was bought at Yangoro Market, Zaria, Kaduna State. It was then taken to the Department of Botany, Ahmadu Bello University, Zaria, Kaduna State, Nigeria where it was identified by a Taxonomist as *Garcinia kola*. Before analysis, the seeds were peeled and cut into tiny pieces in order to increase the surface area needed for the sample to dry quickly. It was then air dried for a week at room temperature and subsequently milled into powder.

Preparation of Plant Extracts

Preparation of Aqueous Extract

The aqueous extract was obtained according to the method describe by Dah-Nouvlessounon *et al.* (2015). Briefly, the powder (49.07 g) of *G. kola* obtained above was macerated into 200 ml of distilled water under magnetic agitator for 72 hours at room temperature. The homogenate was then filtered two times on absorbent cotton and once on Whatman N°1 paper (125 mm ø, Cat No 1001 125). For the aqueous extract, the filtrate was dried using water bath. All extracts were stored in labelled sterile container and kept at -20°C until further use.

Preparation of Ethanol Extract

The extract was made using an adapted methods described by Sanogo *et al.* (2006) and N'Guessan *et al.* (2007). About 49.0 g of *G. kola* extract was macerated in 200 ml of 70% ethanol for 72 hours. The obtained extract was filtered thrice using Whatman N°1 filter paper (125 mm ø, Cat No 1001 125). The filtrate was directly dried using water bath to obtain the ethanolic extract of *G. kola*. The ethanol extracts were stored in labelled sterile container and kept at -20°C.

Determination of Phytochemical Constituents

The extracts were screened for the qualitative presence of carbohydrates, anthraquinones, alkaloids, saponins, flavonoids, cardiac glycosides, tannins and steroids using the methods described by Evan (1996).

Identification of Clinical Isolates

Staphylococcus aureus and *Streptococcus pyogenes* clinical isolates collected were identified based on their cultural, morphological and biochemical characteristics and were sub-cultured onto mannitol salt agar and blood agar respectively for purity. They were maintained on agar slants at -4°C in the refrigerator until when required for use.

Preparation of Stock Solution

Four grams (4g) of the dried aqueous extract of Bitter kola was weighed on a balance. Using a sterile syringe, 10ml of sterile distilled water was added to it and mixed. This formed the stock solution for Bitter kola aqueous extract (400mg/ml) and was stored at -20°C for subsequent analysis. Ethanol extract of Bitter kola were prepared in a similar way to the water extracts as described above, except that the respective extraction solvents was used in place of the sterile distilled water.

Preparation of Different Concentrations

The stock solution was serially diluted with the extraction solvent, in sterile test tubes labelled and arranged from the highest to lowest concentration of extract desired. Double dilution series ranging from 200mg/ml to 25mg/ml were prepared from stock solution. The different dilutions were labelled and kept in sterile bottles for subsequent use.

Preparation of Standard Inoculum

Two to three isolated colonies of the test organisms were picked using an inoculating loop from overnight growth on nutrient agar plates and suspended in sterile saline. This was mixed and the turbidity of suspension was matched with that of the 0.5 McFarland standards which was prepared by mixing 9.4ml 1% of sulphuric acid with 0.6ml 1% barium chloride.

Determination of Antibacterial Activity of *Garcinia kola* against the Test Organisms

Each of the standardized inoculum was inoculated on Mueller Hinton Agar plate separately. This was done within 15 minutes

after inoculum was standardized. The plates were inoculated by dipping a sterile swab into the inoculum. Excess inoculum was removed by pressing the swab firmly against the side of the tube above the level of the liquid. The swab was swab over the surface of the medium. This was left to dry for 2-5 minutes at room temperature with the lid closed before extract were applied. The agar well diffusion method was used to determine the antibacterial activity of the *Garcinia kola*. A sterile cork borer was used to create 4 wells of 6mm in the Mueller Hinton agar and a distance of at least 15mm was maintained from the edges of the plates to prevent overlapping of inhibition zones. Then, the wells were aseptically filled with different concentration of the extract using a micro pipette. The concentrations were 200, 100, 50, 25mg/ml. The plates were incubated at 37°C for 24hrs. The plates were examined after 24hrs and diameters of the zones of inhibition were measured in mm.

Determination of Minimum Inhibitory Concentration

Tube dilution method was used. Two fold serial dilution of each extracts that shows the least inhibition against growth of the test organisms was serially diluted using a set of four test tubes containing Mueller Hinton Broth each. The fifth tube contained only broth which served as negative control. About 0.1ml of the inocula was transferred into each of the test tubes except the test tube serving as control. The test tubes were incubated at 37°C for 18- 24 hours. The test tubes were then observed for bacterial growth by the presence of turbidity. The lowest dilution of the bitter kola extracts at which no growth was observed was the minimum inhibitory concentration (Tripathi, 2013).

Determination of Minimum Bactericidal Concentration

Minimum bacterial concentration is the lowest concentration of the sample that kills the organism. This was obtained by streaking out the cultures from the minimum inhibitory concentration test tubes on Mueller-Hinton agar plates. Minimum bactericidal concentrations were indicated by failure of the organism to grow on the media plates after 24 hours incubation period (French, 2006).

RESULTS AND DISCUSSIONS

The increase in failure of many antibiotics for treating bacterial infections has led to the screening of several medicinal plants for their potential antibacterial activity. The phytochemical screening performed on Bitter kola revealed the presence of several secondary metabolites which includes: saponins, alkaloids, flavonoids, glycosides, carbohydrates, anthraquinones, unsaturated steroids, and tannins (Table 1). The presence of these constituents in both ethanol and aqueous extracts could be responsible for the antibacterial activities exhibited by *Garcinia kola*. The presence of these phytochemical constituents in this study also confirmed what other researchers had found in their studies (Ghamba *et al.*, 2011, Esiegwu *et al.*, 2014).

Bitter kola extracts exhibited antibacterial activity against *Streptococcus pyogenes* and *Staphylococcus aureus*. *Streptococcus pyogenes* was most susceptible to ethanol extract of Bitter kola with an inhibition zone of 12 to 24mm, while *Staphylococcus aureus* was most susceptible to aqueous extract of Bitter kola with an inhibition zone of 10 to 28mm (Table 2). There was a significant difference between the diameters of zone of inhibition of bitter kola extracts for each organism. The reason for the difference could be due to rate of diffusion of the extracts, depth of the medium or density of inoculation. The zone of inhibition of 10mm to 28 mm for aqueous and ethanol extracts of *Garcinia kola* in this study could be due to the phytochemical constituents in *Garcinia kola* that had been confirmed to have antibacterial activities. It is comparable to the findings of Ogbulie *et al.* (2007) in Owerri, Nigeria where zone of inhibition was 9 to 21mm and Adegboye *et al.* (2008) in Nigeria also reported zone of inhibition of 10-25 mm for different bacterial in their study which is comparable to our findings of 10-28 mm. The result however is in contrast to the results of Uhunmwangho *et al.* (2014) in Ekpoma, Nigeria, that found the zone of inhibition of both aqueous and ethanol extracts to be 6 to 12mm.

The aqueous extract exhibited activity against *Streptococcus pyogenes* and *Staphylococcus aureus* with minimum inhibitory concentration (MIC) values of 50mg/ml and 25mg/ml respectively, while the ethanol extract showed

activity against *Streptococcus pyogenes* and *Staphylococcus aureus* with minimum inhibitory concentration (MIC) values of 25mg/ml and 12.5mg/ml respectively (Table 3). This is contrary to Akoachere *et al.* (2002) in Cameroon that reported MIC of 0.0005 to 0.449µg/ml. The MIC in this study however is comparable to the found by Ghamba *et al.* (2011) in Nigeria, where the MICs of the *G. kola* extracts varied between 12.5mg/ml and 25mg/ml against all the bacteria isolates used in their study.

The extracts also showed bactericidal activity against both organisms. The minimum bactericidal concentration (MBC) value of aqueous extract for *Streptococcus pyogenes* and *Staphylococcus aureus* was 50mg/ml and 25mg/ml respectively, while the minimum bactericidal concentration (MBC) value of ethanol extract for *Streptococcus pyogenes* and *Staphylococcus aureus* was 25mg/ml (Table 4). This result contrasts that of Akoachere *et al.* (2002) in Cameroon that was 0.9µg/ml-1.11µg/ml. This observed variation of MIC and MBC could be attributed to different harvest processing, drying method and storage condition of the plant.

Table 1: Phytochemical Constituents of Extracts of Bitter Kola

Test	Aqueous	Ethanol
Saponin	Positive	Positive
Alkaloids	Positive	Positive
Flavonoids	Positive	Positive
Glycoside	Positive	Positive
Carbohydrate	Positive	Positive
Anthraquinones	Positive	Positive
Unsaturated steroid	Positive	Positive
Tannins	Positive	Positive

Table 2: Antibacterial Susceptibility of Aqueous and Ethanol Extracts of Bitter Kola on *Streptococcus pyogenes* and *Staphylococcus aureus*

Test Organisms	Extracts	Conc. (mg/ml)	ZIH (mm)
<i>S. pyogenes</i>	Aqueous	200	14
		100	12
		50	10
		25	0
	Ethanol	200	24
		100	20
		50	16
		25	12
<i>S. aureus</i>	Aqueous	200	28
		100	24
		50	18
		25	10
	Ethanol	200	20
		100	16
		50	12
		25	10

Conc = Concentration

ZIH = Zone of Inhibition

Table 3: Minimum Inhibitory Concentration of Bitter Kola Extracts on *Streptococcus pyogenes* and *Staphylococcus aureus*

Test Organisms	Extract	Concentration (mg/ml)
<i>S. pyogenes</i>	Aqueous	50
	Ethanol	25
<i>S. aureus</i>	Aqueous	25
	Ethanol	12.5

Table 4: Minimum Bactericidal Concentration of Bitter Kola Extracts on *Streptococcus pyogenes* and *Staphylococcus aureus*

Test Organisms	Extract	Concentration (mg/ml)
<i>S. pyogenes</i>	Aqueous	50
	Ethanol	25
<i>S. aureus</i>	Aqueous	25
	Ethanol	25

Conclusion

The study evaluated the antibacterial activity of Bitter kola extracts on *Streptococcus pyogenes* and *Staphylococcus aureus* collected from Ahmadu Bello University Teaching Hospital Shika, Zaria. The aqueous extract had better activity on *Staphylococcus aureus* than *Streptococcus pyogenes* and the ethanol extract was more potent against *Streptococcus pyogenes* than aqueous extract. The minimum inhibitory concentration and minimum bactericidal concentration of the extracts against the isolates were found to be 50mg/ml, 25mg/ml, 25mg/ml, 12.5mg/ml and 50mg/ml, 25mg/ml, 25mg/ml, 25mg/ml respectively. This imply that bitter kola can be formulated and used as drugs in the treatment of infections caused by *Streptococcus pyogenes* and *Staphylococcus aureus*

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