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# ANTIBACTERIAL ACTIVITY OF GARCINIA KOLA EXTRACTS AGAINST CLINICAL ISOLATES OF STREPTOCOCCUS PYOGENES IN ZARIA, NIGERIA

***[1]Otori, M.O., [2]Ella, E.E., [2]Shaibu, M.A.,[2]Ochigbo, V., [1]Odeke, E.H., [1]Abe, A.S., [1]Esew, O., [1]Edem, U.O., [1]Idio, U.I., [1]Adudu, J.A., [3]Okezie, V.C. and [1]Ukwuije, N.**

[1]National Research Institute for Chemical Technology, Zaria, Nigeria;[2]Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria; [3]Nigerian National Medicine Development Agency, Lagos, Nigeria.

Corresponding author: otorimercy@yahoo.com 08035984377

## ABSTRACT

*Garcinia kola,* commonly called bitter kola is known mostly for its antimicrobial potentials. Moreover, *Streptococcus pyogenes* a Gram-positive, facultative anaerobe, and non-spore forming bacterium that occur in long chains cocci is unique in causing different bacterial infection, hence a need for alternative therapy. Therefore, this study was carried out to evaluate the antimicrobial activities of *Garcinia kola* seed extracts against clinical isolates of *Streptococcus pyogenes* in Zaria, Nigeria. In this study, *Streptococcus pyogenes* was isolated from throat swabs and sputum of patients with respiratory illnesses attending a selected clinic in Zaria using chocolate agar and further characterized using standard biochemical test. Isolates were stored on agar slants at $4^0$C for further use. Thereafter, aqueous and n-butanol, extraction of dried seeds of *Garcinia kola* was carried out using Soxhlet extraction method. Phytochemical screening of the extract was determined using standard techniques while antimicrobial activities against the stored bacterium were determined using Kirby Bauer method. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using previous standard microbiological technique. Photochemistry of the extract showed the presence of tannin, saponin, alkaloid, phenol, steroids, and flavonoid. Highest (16-28mm) and lowest (6-8mm) antimicrobial activities against the bacteria were observed among n-butanol and aqueous extract respectively while MIC and MBC of n-butanol were 0.31 and 0.63 mg/ml respectively. The antibacterial activities of the extracts observed in this study, justify the use of this plant for the treatment of infections caused by these organism in traditional medicine.

**Keywords:** *Garcinia kola*, Phytochemicals, *Streptococcus pyogenes*, MIC, MBC

## INTRODUCTION

There has been an appreciable interest in research on the bioactivity of natural products for use as herbal medicines (Aladesanmi, 2007) because herbal medicines are cheap and natural with higher safety margins and less or no side effects (Gupta, 2012). According to World Health Organization (2013), up to 80% of the African population use traditional medicine for their primary health care needs. One such product is *Garcinia kola* Heckel most valued and cultivated in many parts of Nigeria for its medicinal properties.

*Garcinia kola*, commonly called bitter kola belongs to the family *Guttiferae* (Farombi and Owoeye 2011; Adesuyi *et al*., 2012). It is known as *Orogbo* in Yoruba land, *Namijin-goro* among the Hausas, *Akuilu* in Igbo land and *Oro* in Ebira land. It is cultivated and distributed throughout West and Central Africa and is known mostly for its antimicrobial potentials (Natural Standard Monograph, 2008). The genus *Garcinia* is divided into different species depending on their geographical distribution and location. These include: *Garcinia kola*, *G. courauana, G. cambogia, G. bractaeta, G. mangostana, G. multiflora, G. neglecta, G. puat, G. pyrifera* and *G. actroviridis* (Natural Standard Monograph, 2008).

Medicinally, the stem, bark and seeds are used among traditional healers to treat acute fever, cough, throat infections, bronchitis, hepatitis, liver disorders and as an anti-vomiting agent (Gill, 1992; Farombi *et al.*, 2005; Farombi and Owoeye, 2011). It is also been reported to provide remedy for treatment of stomach ache and gastritis (Ajebesone and Aina, 2004; Adegboye *et al.*, 2008). Whereas these claims exist, not much has been done to validate them and determine appropriate effective concentrations as well as safe doses to humans. Thus, this work intends to assess the activity of crude extracts of *Garcinia kola* against a respiratory tract pathogen *Streptococcus pyogenes*.

*Streptococcus pyogenes* (group A β-haemolytic *Streptococcus* [GAS]) is a spherical, Gram-positive, non-motile, catalase negative, facultative anaerobe, non-spore forming bacterium pathogen that occurs as long chains of cocci and occasionally in pairs (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, and bacteremia (Mahon *et al*., 2015). The aim of this study was to determine the *in-vitro* potency of *Garcinia kola* extracts on clinical isolates of *Streptococcus pyogenes* in Zaria, Nigeria.

## MATERIALS AND METHODS

### Isolation of *Streptococcus pyogenes* from Sputum and Throat Swabs

Throat swabs and sputum for isolation of *Streptococcus pyogenes* were collected from patients with respiratory illnesses attending Ahmadu Bello University Medical Centre (ABUMC), Samaru, Hajiya Gambo Sawaba General Hospital (GSH), Zaria City and Zaria Clinic and Medical Centre (ZC), Tudun Wada,

Zaria, Kaduna State for a period of 13 months starting from February 2017 to March 2018. Preliminary isolation was performed by streaking the swabs on chocolate agar and incubated in candle jar at 37℃ for 24 hours. After incubation, discrete colonies with clear zones of haemolysis were further observed for their Gram reaction and microscopic morphology (Cheesbrough, 2006). Presumptive colonies were further subjected to a battery of biochemical assay as described by (Cheesbrough, 2006). These include determination of catalase activity and reaction with bacitracin. Isolates were confirmed using a commercial identification kit for Streptococcus (Microgen$^{TM}$ Strep ID, U.K.) in accordance with the manufacturer's instruction.

**Collection and Identification of *Garcinia kola***

One thousand, four hundred grams of the seeds of *Garcinia kola* were purchased from Yangoro, Hanwa in Sabon Gari local government area of Kaduna State, identified and authenticated by a Taxonomist at the Department of Botany, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The voucher number was 279.

**Processing and Extraction of Plant Materials**

*Garcinia kola* seeds were dried at room temperature (30$^{o}$C) and pulverized and subjected to aqueous and ethanolic extraction (Soxhlet extraction) as described by Akerele *et al.* (2007) and Himal *et al*. (2008). The extracts were allowed to evaporate to dryness using rotary evaporator and the dried extracts were stored in air tight bottles.

**Phytochemical Screening**

The extracts were screened for the qualitative presence of some phytochemicals: the presence of tannins was determined following the method of (Sofowora, 1993a). Similarly saponins, phlobatannins, anthraquinones, terpenoids and steroids were assayed using method described by (Sofowora, 1993b). Screening for flavonoids was performed by the method of (Harborne, 1998), while alkaloids, reducing Sugars, glycosides and volatile oils were determined using the method of Talukdar *et al.* (2010).

**Fractionation of Extract of *Garcinia kola***

The *G. kola* extract was fractionated (partitioned) with n-hexane (HN), ethyl acetate (EA), n-butanol (BL) and water) in order of decreasing polarities in accordance with the method described by Akinpelu *et al.* (2009). All the soluble fractions collected were labeled and evaporated to dryness using a rotary evaporator and kept in a refrigerator at 4$^{o}$C for further use.

**Determination of the Antibacterial Activities of the Extracts of *G. kola* against Clinical Isolates of *S. pyogenes***

The bacterial suspension was standardized to match 0.5 McFarland standards (1.5 x $10^8$ cfu/ml) in sterile physiological saline according to the method described by (Cheesbrough, 2006). A 100mg/ml stock solution of each extracts was prepared from which doubling dilutions of 50mg/ml to 12.5mg/ml were prepared from stock solution. The antibacterial activity of each extract n-hexane (HN), ethyl Acetate (EA), n-Butanol (BL) and aqueous (AQ) against clinical isolates of *S. pyogenes* was evaluated using agar well diffusion method. About 100µl of standardized inoculum of each bacterium was inoculated on Mueller Hinton agar plate (in duplicates) and spread evenly with sterile swab sticks. Wells of 5mm size were cut with a sterile cork borer and each was filled with 100µl of crude extract at varied concentrations of 100, 50, 25 and 12.5mg/ml respectively. The plates were left at room temperature for 30 minutes for prediffusion and then incubated for 24 hours at 37$^{o}$C. The diameter of zone of inhibition was measured. Sterile distilled water was used as a negative control and ciprofloxacin as positive control.

**Determination of Minimum Inhibitory Concentrations (MIC) of the Extracts of *G. kola***

The MICs of the extracts against clinical isolates of *S. pyogenes* were evaluated using agar dilution method, following the method described by (Akinpelu and Kolawole, 2004). The following concentrations of extracts; 12.5mg/ml, 6.25mg/ml, 3.125mg/ml and 1.56mg/ml were prepared by doubling dilutions.

Two ml of extract concentration was added to 18ml of Mueller Hinton agar. About 100µl each of a standardized inoculum of each isolate was spread on the surface of the Mueller Hinton agar using sterile cotton swab sticks. The plates were incubated at 37$^{o}$C for 24 hours. However, the lowest concentration of the extract which inhibited the growth of a test organism was recorded as the minimum inhibitory concentration (MIC). Negative controls were Mueller Hinton agar only and Mueller Hinton agar with extract.

**Determination of Minimum Bactericidal Concentration (MBC) of the Extracts of *G. kola***

The MBC of the extracts was determined using method described by Olorundare *et al.* (1992). Samples was taken from plates with no visible growth during the MIC assay and sub-cultured onto freshly prepared Mueller-Hinton agar medium and later incubated at 37$^{o}$C for 48 hours. The MBC was taken as the lowest concentration of the extract that did not allow any bacterial growth on the surface of the agar plates. Results obtained from the laboratory were presented in tables and figures which were analyzed.

## RESULTS

The extracts of *Garcinia kola* contained tannin, saponin, alkaloid, phenol, terpenoids, steroids, reducing sugars, volatile oil, cardiac glycoside and flavonoid as shown in (Table 1).

The antibacterial activity of extracts of *G. kola* against clinical isolates of *S. pyogenes* is presented in Table 2. The n-butanol extract demonstrated highest activity against *S. pyogenes* clinical isolates with mean zones of inhibition ranging between 16.5mm to 28mm while the aqueous extract had the least antibacterial activities on the isolates compared to other extract with mean zones of inhibition ranging between 6 to 8mm.

The MIC and MBC of the extracts of *G. kola* against *S. pyogenes* are presented in Table 3. The n-butanol extract had the lowest MIC of 0.31mg/ml active against the isolates while the extract with the highest MIC on test isolates and by implication least effective was the aqueous extract which was 2.5mg/ml. The MBC of n-butanol extract against *S. pyogenes* was 0.63mg/ml while the aqueous extract had the highest MBC of 2.5mg/ml.

**Table 1: Phytochemical constituents of extracts of *Garcinia kola***

| Phytoconstituents | AQ | EA | HN | BL |
|---|---|---|---|---|
| Tannins | + | + | + | + |
| Saponins | + | + | + | + |
| Alkaloids | + | + | + | + |
| Cardiac glycosides | + | + | + | + |
| Flavonoids | + | + | + | + |
| Anthroquinone | – | – | – | – |
| Phenol | + | + | + | + |
| Terpenoids | + | + | + | + |
| Steroids | + | + | + | + |
| Reducing sugars | + | + | + | + |
| Volatile oil | + | + | + | + |
| Phlobatanin | – | – | – | – |

Key: + = present, - = absent, AQ = Aqueous, EA = Ethyl acetate, HN = n-Hexane, BL = n-butanol

**Table 2: Susceptibilities of *S. pyogenes* isolates to varying concentrations of the extracts of tested**

| Extracts Tested (mg/ml) | **Mean Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | 100mg/ml | 50mg/ml | 25mg/ml | 12.5mg/ml |
| Aqueous | 8.0 | 6.4 | 6.0 | 6.0 |
| n-Butanol | 28.0 | 23.0 | 19.5 | 16.5 |
| Ethyl Acetate | 22.0 | 20.0 | 16.0 | 12.0 |
| n-Hexane | 18.0 | 16.7 | 13.8 | 12.0 |
| **Mean Zone of Inhibition of Antibiotic (mm)** | | | | |
| Ciprofloxacin (5 µg) | 14.40 | | | |

** = Mean of 42 organisms

**Table 3: Minimum inhibitory concentrations and minimum bactericidal concentrations of various extracts against *S. pyogenes***

| Extracts Tested (mg/ml) | MIC (mg/ml) | MBC (mg/ml) |
|---|---|---|
| Aqueous | 2.50 | 2.50 |
| n-Butanol | 0.31 | 0.63 |
| Ethyl acetate | 0.63 | 1.25 |
| n-Hexane | 0.63 | 1.25 |

## DISCUSSION

The use of plants in medicines has received global recognition due to reduced cost and fewer side effects as compared to the use of orthodox medicine.

The extracts of *G. kola* revealed the presence of tannins, saponins, alkaloids, glycosides, flavonoids, phenol, terpenoids, steroids, reducing sugars, and volatile oil. The presence of these constituents could account for the antibacterial activities exhibited by *Garcinia kola*. The presence of these phytochemical constituents in this study is in agreement with the findings of Ebana *et al.* (1991), Akoachere *et al.* (2002), Esimone *et al.* (2007), Ghamba *et al*. (2011) and Esiegwu *et al*. (2014) and justifies its use as claimed traditionally. The antimicrobial properties of the *G. kola* seeds are attributed to flavonoids (Anegbeh *et al*., 2006). 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 scanvenge for hydroxyl radicals and superoxide anion radicals and thus health promoting in action (Ferguson, 2001).

Cardiac glycoside also found in *Garcinia kola* is used for treatment of *S. pyogenes* infections such as cough and chest pain in many areas of Western Nigeria especially the Yoruba speaking communities. Tannin is known to exert antimicrobial activities by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells (Scalbert, 1991). Motar *et al*. (1985) reported that tannin is useful in treatment of inflamed or ulcerated tissues and of course *Streptococcus pyogenes* infections like pharyngitis and tonsillitis that are inflammation of pharynx and tonsil can be treated using tannin. Tannins could induce local vasco-constriction in small mucous (Cole, 1992). Just *et al.* (1998) revealed inhibitory effect of saponins on inflamed cells.

These phytochemicals were found in all the extracts: however, the n-butanol had the highest action with a zone of inhibition of 28mm/ml and an MIC of 0.31mm/ml. The n-butanol demonstrated highest activity against *S. pyogenes* clinical isolates while the aqueous had the least antibacterial activities on the isolates. The result indicates that the extracts demonstrated good inhibitory activities against the isolates which could be attributed to their polarities. The n-butanol extract performed better than the standard control drugs used in this study. It had higher activities (zone of inhibitions) than the antibiotic ciprofloxacin.

The n-butanol extract demonstrated the lowest MIC and MBC against the isolates which could be an indication of the activity of the constituents in it such as tannins, saponins, flavonoids, alkaloids and glycosides. It is therefore possible that n- butanol separated more quantity of the active compound than the other

solvents. The ethyl acetate and n- hexane had similar activities but were not as active on the bacterial isolated as the n-butanol. There is dearth of information on the fractionation of *G. kola* extracts against *S. pyogenes*. Compounds from the plant have also proved effective against some strains of flu, a contagious respiratory disease also called influenza (Iwu, 1993). The report of the findings of Ogbulie *et al*. (2007) in Owerri, Nigeria and Uhunmwangho *et al*. (2014) in Ekpoma, Nigeria also confirmed that *G. kola* had antibacterial activity against *S. pyo*genes. Adegboye *et al*. (2008) in Nigeria and (Seanego, 2012) in South Africa also reported antibacterial activity of *G. kola* against different bacteria in their study.

## CONCLUSION

Based on the observations made in this study, the following conclusions were drawn:

The phytochemical constituents of *Garcinia kola* extracts revealed the presence of tannins, saponins, alkaloids, cardiac glycosides and flavonoids among others.

The extracts of *G. kola* showed significant antibacterial activities on *S. pyogenes* at different concentrations. However, the n-butanol extracts had more antibacterial activities and the antibacterial susceptibility of the extracts increases as the concentrations increases. The MIC of the extracts ranges from 0.31mg/ml to 2.5mg/ml while the MBC were found to be 0.63 to 2.5mg/ml. Therefore, the extracts were bacteriostatic at lower concentration and bactericidal at higher concentration.

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