# ANTIBIOGRAM OF *Garcinia kola* SEEDS EXTRACT ON SOME SELECTED ENTERIC BACTERIA

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Abstract: *Garcinia kola* is a plant of Western and Central African origin and has been found to be medicinally important. Antibacterial activity of aqueous and methanol extracts of the plant was conducted against Isolates of *Klebsiella pneumoniae, Shigella* species and *Salmonella typhi.* The seed extracts were prepared by soaking 35 g of powdered seeds in 135 ml of methanol and 21 g in 100 ml of sterile distilled water and processing to produce 1.40, 0.70 and 0.35 mg/ml for aqueous and 2.50, 1.25 and 0.625 mg/ml for methanolic extract. Well diffusion method was used to test extracts against organisms and minimum inhibitory concentration (MIC) was obtained from turbidimetry. The test organisms were found to be more susceptible to methanol extract than the aqueous extract; methanol extract showed antibacterial activity only against *K. pneumonia*e and *Shigella* species but was ineffective against *S. typhi.* The MIC of aqueous extract of *G. kola* seeds onall the test organisms was 1.40 mg/ml. The MIC of methanol extract was found to be at 1.25 mg/ml for *K. pneumoniae* and *Shigella* species. The minimum bactericidal concentrations (MBC) of both aqueous and methanol seed extracts in the test organisms were also examined. Methanol extract showed bactericidal effects on *K. pneumoniae* and *Shigella* species at the same concentration of 2.5 mg/ml. Therefore, the extracts found to be effective to the test organisms should be considered by pharmaceutical industry for the production of antibacterial agents for the treatment of diseases caused by the test organisms and other enteric species.

**Keywords:** Aqueous extract, *Garcinia kola*, methanol extract, MIC, MBC, sensitivity

## Introduction

Medicinal plants are of great importance to the health of individuals and communities. Plants remain the basis for development of modern drugs for the preservation of health in the rural and urban communities worldwide in this present technological era (Earnest and Ekene, 2014). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants.

Traditional herbalists in Nigeria use a variety of herbal preparation to treat different kinds of ailments such as gonorrhea, sore throat and skin infections like eczema. This has been the case ever before the introduction of antibiotics and other modern drugs into Africa (Faleyimu and Oluwalana, 2008). *Garcinia kola* (bitter kola) belongs to the Clusiaceae/Guttiferae family and is mainly found in the tropical forests of Central and West Africa. The plant is used in folklore remedies for diseases such as liver disorder, diarrhea, laryngitis, bronchitis, and gonorrhea (Nweke *et al*., 2019). It is commonly referred to as bitter kola for its bitter taste and has the popular acronym "wonder plant" amongst the southwestern Nigerian people because every part of it has been found to be of medicinal importance (Earnest and Ekene, 2014). *G. kola* is a perennial dicotyledonous plant growing in the forest, distributed throughout West and Central Africa and commonly referred toin Nigerian languages as "*Namijingoro*" (Hausa), "*Agbilu*" (Igbo) (Esemonu *et al*., 2005; Nweke *et al*., 2019), and *"orogbo"* in (Yoruba) (Ndukwe *et al*., 2005; Nweke *et al*., 2019). It is also called bitter cola and male kola due to the reported aphrodisiac properties and has economic value across West African countries where the seeds are commonly chewed and used for traditional ceremonies (Eleyinmi *et al*., 2006). It is a medium sized evergreen tree, about 15-17 m tall.

The leaves are simple, 6 – 14 cm long and 2-6 cm across. The fruit is a drupe of 5-10 cm in diameter and a weight of 30-50 g (Juliana *et al.*, 2006). The plant's nut contains a high proportion of tannins and guttiferin and studies have shown G. kola to have a neuroprotective effect, sexual enhancement, hepatoprotective activity, and increase in testosterone production due to its antioxidant properties (Nweke *et al*., 2019). The seeds are chewed as an aphrodisiac or used to cure cough, dysentery, chest colds, liver disorders, diarrhoea, laryngitis, bronchitis, and gonorrhea, it is also used to prevent and relieve colic; it can also be used to treat headache, stomachache and gastritis (Udenze *et al.*, 2012). It is also reported that Garcinia species contain mainly flavonoids of the biflavonoid type; these flavonoids are thought to be responsible for the antihyperglycemic activity of its seeds (Katemo *et al*., 2018).

In Sierra Leone, the roots and bark are taken as a tonic for sexual dysfunction in men, it is added into palm wine to improve its potency, the stem bark is used as a purgative and for the treatment of malignant tumors and the sap is used for parasitic skin disease while the latex (gum) is used internally for gonorrhea treatment. It is applied externally to treat fresh wounds (Eleyinmi *et al*., 2006). The plant has also been employed in the treatment of liver disorders while the fruit yield favorite bitter chewing sticks sold in small bundles in local markets across West Africa (Adegboye *et al*., 2008). *Klebsiella pneumonia*e is known to be a major threat to public health, it is the most common factor of nosocomial and community acquired infections (Candan and Aksoz, 2015). It causes a small proportion (1%) of bacterial pneumonia and can produce extensive hemorrhagic necrotizing consolidation of the lung. It produces urinary tract infection and bacteremia with focal lesions in debilitated patients (Brooks *et al*., 2013).

The members of the genus *Shigella* species are important causative agents of bacillary dysentery in human beings and *Salmonellatyphi* is a member of the salmonella serovars that causes enteric fever (Bhatia and Ichhpujani, 2008). Reported developments of antimicrobial resistance in pathogens such as the enteric bacteria has is one of the important issues confronting the health systems around the globe, hence the need to conduct antibiotic assay to ascertain the activity of biter cola on some representatives of the group in contribution to reduction of multiple drug resistance.

## Materials and Methods

### *Study area*

This research was conducted at the Microbiology Laboratory, Department of Microbiology, Federal University Wukari, Taraba State, Nigeria.

### *Collection of plant material*

Fresh seeds of *Garcinia kola* were purchased from New Market Wukari, Taraba State, Nigeria. It was checked and certified by the Department of Botany, Federal University Wukari.

### *Preparation of extracts*

The seeds were peeled and cut into pieces and then dried in the hot-air oven at 40ºC until the constant weight of the seeds was obtained. The seeds were then milled to a fine powder with the aid of a Master Chef Blender (Model MC-BL 1444) and stored in an air-tight container until use. Exactly 35 g of the powdered seeds was soaked in 135 ml of methanol and 21 g in 100 ml of sterile distilled water and shaken intermittently at 120 rpm for 72 h and then filtered to obtain the methanolic and aqueous extracts, respectively. The methanolic (138 ml) and aqueous (127 ml) extracts were evaporated using Thermostat Water Bath (Model HH-6) and both crude extracts were obtained (Adegboye *et al*., 2008).

### *Test organisms*

The organisms used for this research were clinical samples obtained from the National Veterinary Research Institute, Vom, Plateau State, Nigeria. They were confirmed by sub culturing on MacConkey agar *Klebsiella pneumoniea* and *Salmonella/Shigella* agar (SSA) (*Shigella* species and *Salmonella typhi*) and identified based on growth, cultural characteristics and biochemical reactions as done by Maschinen *et al.* (2005).

### *Antimicrobial activity*

The sensitivity testing of the plant extract was determined using agar-well diffusion method as described by Irobi *et al*. (1994), Baur *et al*. (1996) and Maschinen *et al*. (2005) which has been widely used for antimicrobial susceptibility testing. The organisms were first seeded in nutrient broth using McFarland standard and incubated for 18 h before use. The isolates were later sub-culture on Mueller-Hinton agar. Wells were then bored into the agar medium using a sterile 6 mm cork borer. The wells were then filled up with the solution of the extract and care was taken not to allow the solution to spill to the surface of the medium. Ampicillin was used as positive control since all the test organisms were sensitive to it. The plates were allowed to stand on the laboratory bench for between 1 and 2 h to allow proper inflow of the suspension (extracts) into the medium. The plates were incubated at 37ºC for 24 h. The plates were then observed for inhibition zones. The effects of the extract on bacterial isolates were compared to those of the standard antibiotic. The diameter of inhibition zones was measured in millimeter using meter rule.

### *Determination of the minimum inhibitory concentration (MIC)*

The minimum inhibitory concentration was determined by diluting the initial concentration of the extracts using double fold serial dilution (a two-fold serial dilution decreases the concentration of a solution by a factor of two that is, decreases the original concentration by one half) by adding 1 ml of distilled water to 1.40 mg of aqueous extract to obtain concentration of 1.40 mg/ml. The above process was repeated two times to obtain other dilutions; 0.70 and 0.35 mg/ml and the same procedure was followed to obtain 2.50, 1.25 and 0.625 mg/ml concentrations for the methanolic extract (Ibekwe *et al*., 2001). Having obtained the different concentrations of the methanolic and aqueous extracts, 5 ml of sterile peptone water was added to each concentration and inoculated with 0.1 ml of the bacterial suspension (cells that were determined using McFarland standard) and incubated at 37ºC for 24 h. The growth of the inocula in the broth was indicated by turbidity or cloudiness of the broth. The lowest concentration of the extract which inhibited the growth of the test organism was taken as the minimum inhibitory concentration (MIC).

### *Determination of the minimum bactericidal concentration (MBC)*

The Minimum Bactericidal Concentration (MBC) was determined by selecting tubes that showed no growth during MIC determination. A loopful from each tube was sub-cultured onto extract-free freshly prepared nutrient agar plates and was incubated for2 4 h at 37ºC. The least concentration at which no growth was observed was noted as the MBC. Readings were taken in mg/ml (Garba *et al.*, 2013).

## Results and Discussion

This research revealed that aqueous and methanol crude extracts of *Garcinia kola* seed possesses antimicrobial activities against *Klebsiella pneumoniae*, *Shigella* species and *Salmonella typhi* as presented in Table 1. Crude methanol extract showed higher antibacterial activity against *Klebsiella pneumonia*e and *Shigella* species but less effective against *Salmonella typhi.* All the organisms were sensitive to the aqueous extract. The antibiotic control (ampicillin) was more effective against *Klebsiella pneumonia* with diameter of zone of inhibition 27 mm, followed by 24 mm for *Salmonella typhi* and 21 mm for *Shigella* species. The inhibitory effect of aqueous and methanol extract is as thus, 9 and 10 mm for *Klebsiella pneumoniae*, 12 and 13 mm for *Shigella* species, respectively. The inhibitory effect of aqueous extract against *Salmonella typhi* was 11 mm but no inhibition was observed with the methanol extract. The highest activity of aqueous and methanol extracts was found on *Shigella* species 12 and 13 mm, respectively.

**Table 1: Antibacterial activity of the plant seed crude extracts**

| Organisms | Diameter zone of inhibition ± SD (mm) | | |
|---|---|---|---|
| | Aqueous extracts | Methanol extracts | Control (Ampicillin) |
| *Klebsiella pneumoniae* | 9.0±0.4 | 10.00±0.8 | 27.0±0.6 |
| *Shigella* species | 12.0±1.0 | 13.00±0.5 | 21.0±0.2 |
| *Salmonella typhi* | 11.0±0.6 | 0.00±0.0 | 24.0±0.4 |

Table 2 presents the minimum inhibitory concentration (MIC) of aqueous extract of *G. kola* seed on the test organisms. No growth was found at aqueous concentration of 1.40 mg/ml (MIC), but found in the other concentrations in all the test organisms.

**Table 2: MIC of aqueous extract of the studied seedagainst the test organisms**

| Concentrations (mg/mL) | Test organisms | | |
|---|---|---|---|
| | *S. typhi* | *K. pneumonia* | *Shigella* species |
| 1.40 | – | – | – |
| 0.70 | + | + | + |
| 0.35 | + | + | + |

– = absence of growth; + = presence of growth

**Table 3: MIC of methanol extract of *G. kola* seed against the test organisms**

| Concentration (mg/mL) | Test organisms | |
|---|---|---|
| | ***K. pneumoniae*** | ***Shigella* species** |
| 2.50 | – | – |
| 1.25 | – | – |
| 0.625 | + | + |

– = absence of growth; + = presence of growth

Table 3 shows the MIC of methanol extract of the seed on the test organisms, no growth was found at concentrations of 2.50 mg/ml and 1.25 mg/ml for both *K. pneumoniae* and *Shigella* species indicating 1.25 mg/ml as the MIC.

Table 4 indicates the minimum bactericidal concentration (MBC) of both the aqueous and methanol extracts of the plant seed on the test organisms. Methanol extract showed bactericidal effects on *K. pneumoniae* and *Shigella* species at the same concentration (2.50 mg/mL).

**Table 4: Minimum bactericidal concentration (MBC) of aqueous and methanol seed extracts**

| Test organisms | MBC (mg/ml) | |
|---|---|---|
| | Aqueous extract | Methanol extract |
| ***K. pneumonia*** | 0.00 | 2.50 |
| ***Shigella* species** | 0.00 | 2.50 |
| ***S. typhi*** | 0.00 | 0.00 |

Antimicrobial activity of aqueous and methanol extracts of *Garcinia kola* seeds against *Klebsiella pneumoniae*, *Shigella* species and *Salmonella typhi* was determined in this study. Crude methanolic extract showed higher antibacterial activity against *K. pneumonia* with zone of inhibition of 10.00±0.8 mm and *Shigella* species with zone of inhibition of 13.00±05 but was inactive to *S. typhi* as presented in Table 1. Which is in line with the finding of Indabawa & Arzai, (2011), Penduka *et al*. (2011), Akinnibosun and Itedjere (2013), Sanda *et al.*, (2013), Badger-Emeka *et al.* (2018) and Enemchukwu *et al.* (2019) that showed higher activity of alcoholic extract of *Garcinia kola* against enteric bacteria at various concentration. The antibiotic control (ampicillin) was also effective against the test organisms.

The highest MIC inhibitory activity was recorded from the methanolic extract of *Garcinia kola* seeds against *K. pneumonia*e and *Shigella* species (1.25 mg/ml) as shown in Table 3, which is consistent with thef indings of Ezeigbo *et al*., (2016) who confirmed the antibacterial activities of methanolic and aqueous extracts of *Garcinia kola* seeds on *Shigella* species, *Salmonella* species and other bacteria. It is also in conformity with the antibacterial activities of methanol and aqueous seeds extracts on *Klebsiella pneumoniae*, *Salmonella typhi* and other bacteria by Indabawa and Arzai (2011) and Li *et al*. (2012). The MIC for alcoholic extract was found to be 2.5 mg/ml by Akerele *et al.* (2008) and 3.125 by Enemchukwu *et al.* (2019) which are higher than the finding of this research, this may be due to concentration of the solvent.

*Salmonella typhi* was found to be unsusceptible to the ethanol extract of the studied seed which is in concordance with Indabawa and Arzai (2011), this deserves attention for further research since its aqueous extract showed antibacterial effect, the insensitivity may be due to the concentrations of the extract, media used, pH, and inoculum concentration.

A minimum bactericidal concentration (MBC) of 2.5 mg/ml was found with *K. pneumonia*e and *Shigella* species (Table 4) which is in line with the findings of Christinah and Roland (2012), Nas *et al*. (2018)

The methanolic extract of the seeds was more sensitive against the test organisms than aqueous extract. This could be because methanol is more bioactive compared to water and therefore possess higher ability to dissolve more of the active substances from the plant seed than water. All the organisms were susceptible to the aqueous extract.The result agrees with the finding of Okigbo & Mmeka, (2008) and Okwulehie *et al.* (2017). The result contradicts that of Arekemase *et al.* (2012) that found zones of inhibitions from 17 to 23 mm for ethanol and 20 to 27 mm for aqueous (hot water) extracts, it may be because cold water was used in this research.

## Conclusion

Aqueous and methanol extracts of *Garcinia kola* seeds had antibacterial activities on *Klebsiella pneumoniae*, *Shigella* species and *Salmonella typhi*. Test plants thereforecould be used for the treatment of infections caused by the susceptible organisms such as cough, throat infections, typhoid fever, and gastroenteritis. It is also important to standardize the extract and evaluate the antimicrobial efficacy against a wider variety of pathogenic species to obtain a precise therapeutic potential of the plant.

## Conflict of Interest

Authors have declared that there is no conflict of interest reported in this work.

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