International Journal of Research (IJR)
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Vol. 9 Issue 02
February 2022

Antibacterial Activity of Aqueous and Ethanoic Extracts of *Garcinia kola* on *Enterobacter braakii* and *Citrobacter cloacae*

[1,2]Yandev, D., [2]Amos, T., [2]Ishuwa, M.N., [3]Akosu, D.D. and [4]Olasan, J.O.

[1]Microbiology Department, University of Nigeria Nsukka, Enugu State, Nigeria
[2]Microbiology Department, University of Mkar, Mkar, Benue State, Nigeria
[3]Faculty of Life Science, University of Benin, Nigeria
[4]Department of Botany, University of Agriculture Makurdi, Nigeria
Corresponding authors: Yandev, D. (yandevdoowuese@gmail.com); Akosu, D.D (demekaaakosu@gmail.com)

ABSTRACT
*Citrobacte*r and *Enterobacte*r infections are among diseases of public health concern. This study sought to evaluate the possible antimicrobial activity of aqueous and ethanolic extracts of *Garcinia kola* on the two test organisms. Collected plant materials were extracted and tested on the isolates following standard microbiological practices. *Citrobacter clocae* was inhibited more than *E. braakii* at all levels of the aqueous extracts. Similar effect was produced in ethanolic extracts except 20mg and 60mg where higher ZIs were recorded in *E. braakii*. Generally, higher ZIs (>10mm) were observed on *C. clocae* in both aqueous and ethanolic extracts than on *E. braakii*. Aqueous extract had more inhibitory effect on *Citrobacter clocae* than the ethanolic extracts. Ciprofloxacin antibiotic gave higher inhibitory effect on *C. cloaca* than the bitter cola extracts. Bitter cola extracts showed inhibitory properties on the two test organisms under different extracts. *Citrobacter cloaca* was susceptible to both aqueous and ethanolic extracts of bitter cola at all levels of treatments. *Enterobacter braakii* tend to be more resistant to the plant extract than the other test organism due to the low zone of inhibition observed especially under aqueous extract of bitter cola. Comparatively, aqueous bitter cola extract had more inhibitory effect on *Citrobacter clocae* than the ethanolic extracts but ethanolic extracts had more inhibitory effect on *Enterobacter braakii* than the aqueous extract. Thus, when properly optimized in pharmacological research, bitter cola could be explored as a cheap source of antimicrobial agent in the treatment of bacterial infections.

Key words: Antibiotics, *Citrobacte*r, *Enterobacte*r, *Garcinia kola*, Inhibition

INTRODUCTION
Garcinia kola commonly known as bitter kola is a dicotyledonous plant belonging to the family of plants called Guttiferae. In Nigeria, it is common in the South Western States and Edo State (Otor, *et al*., 2001). It plays an important role in African ethnomedicine and traditional ceremonies. It is commonly called "Agbilu" in Igbo land and "Namijin goro" in Hausa lands and "Orogbo" in Yoruba lands of Nigeria (Adegboye *et al.*, 2008). Medicinal plants have been used for the treatment of several human diseases over the century and have been very important in the health care delivery of every nation at one stage or the other (Oluma *et al.*, 2004). There are physical or chemical agents that either kill or inhibit the growth of microorganisms. They could be in form of physical or chemical agents such as temperature, radiations, waves, disinfectants,

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Vol. 9 Issue 02
February 2022

antiseptics, synthetic chemotherapeutic agents, antibiotics and phytotherapeutic agents (compounds derived from plants with medicinal value). They are widely employed to reduce microbial load on animate and inanimate surfaces or in the cure of diseases associated with microorganisms mostly bacteria and fungi. The action of these agents could be either irreversibly inhibiting the growth of bacteria and be termed "bactericidal" or reversibly inhibiting the growth of a microorganism due to continuous contact with the agent and being referred to as being "bacteriostatic" (Rajesh and Rattan, 2008).

For a long while chemotherapeutic agents have been used to combat microbial diseases but even they are becoming less effective as on an alarming rate (Dengat and Shalom, 2010). The emergence of antimicrobial resistance has become a common phenomenon and it is threatening (Maxwell *et al.,* 2000), or has threatened the efficacy of chemotherapeutics (Abiola and Bamidele, 2015). Microorganisms that develop antimicrobial resistance are sometimes referred to as "superbugs (Greenwood, 2007). As a result, the medicines become ineffective and infections persist in the affected body system, increasing the risk of spread to other individuals. The development of multiple antibiotic resistant organisms has constituted a global problem as far as treatment of some infectious diseases is concerned and infectious diseases still remain significant causes of morbidity and mortality in man especially in the developing countries. Drug reaction and side effects, increased risk of malignancy and the adulteration of drugs (Greenwood, 2007) have joined antimicrobial resistance to further reduce the overall effectiveness of chemotherapeutics in the fight against microbes.

Recent research has focused on natural plant product as alternatives to the existing drugs for disease remedy in developing countries (Aiyegoro *et al.*, 2007). Plant derived medicines have been part of traditional health care in most parts of the world for ages and there is increasing interest in them as sources of agents to fight microbial diseases (Mohana *et al.*, 2008; Ajayi and Akintola, 2010). In many parts of the world, including Nigeria, herbal medicine practitioners are still consulted as a first choice in the treatment of ailments, due to the fact that traditional medicine blends readily with the socio-cultural life of the people, and the fact that orthodox medicine are more expensive to procure and some orthodox pharmaceutical preparations are many times faked (Amuse *et al.*, 2011). There is a vast array of medicinal plants that could be used singly or in combination with other medicinal plants to confer synergistic effects in the treatment of ailments or even serve as principal raw material for the production of other conventional medicines (Tahir and Khan, 2012).

A good example is the bitter kola (Garcinia-kola), a tropical plant of the African continent which has been the subject of investigation as a potential source of numerous antimicrobial compounds (Madubunyi, 2015). Depite having been referred to as the wonder plant because almost every part of it can be used for medicinal purpose (Han *et al.*, 2005), the seeds are by far the most important products of the plant and they are at amongst the most traded non-timber forest products (Dranca and Oroian, 2016). When chewed, they have a bitter astringent and resinous taste, somewhat resembling that of raw coffee bean (Hutchinson and Danktur, 2004) with slight residual sweetness (Mshui, 2000). The seed of Garcinia kola is edible and it is very much valued in Nigerian homes as a substitute for the true kola nuts (*Cola nitidatis*). *Citrobacte*r and *Enterobacte*r infections are among diseases of pubic health concern. Treatment is difficult due to

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Vol. 9 Issue 02
February 2022

the highly resistant nature of the organisms to antibiotics. This study sought to evaluate the possible antimicrobial activity of **a**queous and ethanoic extracts of *Garcinia kola* on *Enterobacter braakii* and *Citrobacter cloacae*.

MATERIALS AND METHOD

Collection of plant materials

The plant materials used for this study were the seeds of Garcinia kola (bitter kola nuts). They were obtained from the Juma'at mosque in Hausa quarters located in Gboko metropolis of Gboko, Benue State

Preparation and extraction of plant materials

Plant materials collected were washed under running tap water after the husks have been removed. The washed plant materials were air-dried under shade for two weeks. The dried plant materials were pulverized mechanically using mortar and pestle. The plant materials were extracted following procedures described by Sanyoku (2018). Absolute ethanol were used to soak the pulverized plant materials. The mixture were allowed to stand for 72 hours with intermittent shaking. After 72 hours the mixture were filtered and the filtrate was dried using water bath. The dried filtrate were reconstituted using 10% v/v ethanol as a solubility agent. The concentration of the filtrate were in five different concentrations viz: 100mg, 80mg, 60mg, 40mg and 20mg per mL,

Test organisms

The test organisms for this study were *Citrobacter cloacae*, and *Enterobacter braakii*. The stock culture maintained in McConkey agar was obtained from the Bacteriology Department of National Veterinary Research Institute, Jos, Plataeu State.

Preparation of inoculum

A loopful of each test organism were taken and sub-cultured in test tubes containing 10ml of MeConkey broth. The test tubes were incubated at 37°C for 24hours. The broth were standardized using sterile normal saline to obtain a population of 10° cfu/mL.

Experimental design

Five different concentrations of the plant extracts were prepared using 10% v/v ethanol as the solvent. 10% v/v ethanol were used as the negative control and ciprofloxacin; an antibiotic were used as the positive control. The experiments was carried out in triplicates. A plate inoculated with a single test organism was tested with a single concentration three times.

Antimicrobial activity of *Garcinia kola* on test organisms

To test the antimicrobial activity of Garcinia kola against the test organisms, disc diffusion technique was applied following the Kirby-Bauer method as described by Prescott *et al.*, (2005). Following their description, circular discs cut from filter paper were sterilized in hot air oven for 60 minutes. The sterilized discs was impregnated with the plant extracts and the controls by

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Vol. 9 Issue 02
February 2022

soaking the discs in different concentrations of the plant extracts and the respective controls. 1 mL of each test organism were placed on already prepared media plates (McConkey agar were used) and spread evenly. Sensitivity discs containing either the plant extracts or the controls were placed on the agar plate using sterile forceps. The plates were labeled properly. The plates were incubated at 37°C for 48 hours. The incubated plates were observed for possible zones of inhibition. The zones of inhibitions were measured and recorded.

Statistical Analysis

The data collected from this study was subjected to Chi square and T-test analysis using the Minitab software (version 16) at 95% confidence limit. Data were presented in tables and graphs.

RESULTS AND DISCUSSION

Table 1 gives the results of qualitative tests of *Citrobacter clocae* susceptibility by aqueous bitter cola extracts from three different culture plates. Only one out of three plates (33.3%) showed visible signs of bacterial inhibition at 20mg and 40mg of aqueous bitter cola extracts. Inhibitions were observed in two of the three plates (66.7%) using 80mg and 100mg of the treatment. All the three culture plates (100%) showed visible signs of *C. clocae* inhibition under 60mg of aqueous bitter cola extracts. Table 2 shows the exact measurements of zones of inhibition (ZIs) of *Citrobacter clocae* of aqueous bitter cola extracts in three culture plates. In Plate 1, the ZIs were 12mm and 14mm at 60mg and 80mg treatment concentrations respectively. In Plate 2, the ZIs were 10mm in both 80mg and 100mg of treatment. In Plate 3, the ZIs were 15mm at 40mg and 100mg of treatment. Therefore, 20mg aqueous bitter cola extracts gave the lowest ZI (10mm) while 40mg and 100mg each gave the highest ZIs (15mm). All observed ZIs values were below those of the control values of Ciprofloxacin (20mm) and Ethanol (16mm).

Table 3 gives the results of qualitative tests of *Citrobacter clocae* susceptibility by ethanolic bitter cola extracts from three different culture plates. Only one out of three plates (33.3%) showed visible signs of bacterial inhibition at 20mg and 60mg of ethanolic extracts. Inhibitions were observed in two of the three plates (66.7%) using 40mg and 80mg of the treatment. All the three culture plates (100%) showed visible signs of *C. clocae* inhibition under the highest concentration of 100mg of the ethanolic extracts. Table 4 shows the exact measurements of zones of inhibition (ZIs) of *Citrobacter clocae* of ethanolic bitter cola extracts in three culture plates. In Plate 1, the ZIs were 12mm and 15mm at 80mg and 100mg of treatments respectively. In Plate 2, the ZI was 14mm at 100mg while Plate 3 recorded ZIs of 12mm and 13mm at 80mg and 100mg of treatments respectively. The overall result showed that, 20mg and 60mg had the lowest inhibitory power (7mm and 6mm respectively) followed by 40mg with ZI of 10mm. At higher concentration of the ethanolic extracts, zones of inhibition increased to 12mm at 80mg and also 15mm at 100mg. However, all observed ZIs values were below those of the control values of Ciprofloxacin (20mm) and Ethanol (16mm).

The effects of the two extracts used are compared in Table 5. All plates showed signs of *Citrobacter clocae* inhibition in 60mg of aqueous extracts and 100mg of ethanolic bitter cola extracts. Aqueous extracts at 40mg and 100mg gave the highest ZIs (15mm) while 100mg of ethanolic extracts gave the highest ZI (15mm). Table 6 gives the results of qualitative tests of

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Vol. 9 Issue 02
February 2022

Enterobacter braakii susceptibility by aqueous bitter cola extracts from three different culture plates. None of the plates showed signs of *E. braakii* at 20mg, 40mg and 100mg of aqueous bitter cola extracts. Only one out of three plates (33.3%) showed visible signs of bacterial inhibition at 60mg and 80mg of the treatment. Table 7 gives the exact measurements of zones of inhibition (ZIs) of *Enterobacter braakii* in aqueous bitter cola extracts in three culture plates. In Plate 1, the ZIs were 6mm and 5mm at 60mg and 80mg treatment concentrations respectively. In Plate 2 and 3, there was no inhibition. Therefore, ZI was Zero at 20mg, 40mg and 100mg of aqueous bitter cola extracts while the highest ZI of 6mm was recorded in 60mg of treatment. The observed differences among the five treatment levels as regard the highest zones of inhibition recorded are statistically significant (χ^2 = 16.72, P=0.002, P<0.05). All observed ZIs values were far below those of the control values of Ciprofloxacin (20mm) and Ethanol (10mm).

Table 8 gives the results of qualitative tests of *Enterobacter braakii* susceptibility by ethanolic bitter cola extracts from three different culture plates. None of the plates showed signs of *E. braakii* at 80mg of ethanolic bitter cola extracts. At least one out of the plates showed signs of inhibition in other concentrations while all plates (100%) showed observable signs of inhibitions at 100mg of the treatment concentration. Table 9 gives the exact measurements of zones of inhibition (ZIs) of *Enterobacter braakii* in ethanolic bitter cola extracts in three culture plates. In Plate 1, the ZI was Zero in all treatment concentrations except at 100mg with 6mm ZI. In Plate 2, ZIs were 10mm at 20mg and 7mm at 100mg of extracts. In plates 3, ZIs were 8mm at 20mg and 40mg of extracts while other concentrations gave lower ZIs. Therefore, the highest ZI of 10mm was recorded in 20mm ethanolic treatment followed by 8mm recorded in 40mg of treatment. All observed ZIs values were below the Ciprofloxacin (20mm) value. However, 20mg of ethanolic bitter cola had the same effect as the Ethanol control as both gave 10mm ZIs. Table 11 compares the zones of inhibition of the two test bacteria (*Citrobacter clocae* and *Enterobacter braakii*) under the two types of bitter cola extracts. *Citrobacter clocae* was inhibited more than *E. braakii* at all levels of the aqueous extracts. Similar effect was produced in ethanolic extracts except 20mg and 60mg where higher ZIs were recorded in *E. braakii*. Generally, higher ZIs (>10mm) were observed on *C. clocae* in both aqueous and ethanolic extracts than on *E. braakii*. Aqueous extract had more inhibitory effect on *Citrobacter clocae* than the ethanolic extracts.

The present results have also shown that susceptibility of microorganisms to plant products used depend on the type of microorganism tested, the extract concentrations and methods of extraction used. For example *Citrobacter cloaca* was susceptible to both aqueous and ethanolic extracts of bitter cola at all levels of treatments. Ciprofloxacin antibiotic gave higher inhibitory effect on *C. cloaca* than the bitter cola extracts. This could be due to the refined nature of the drug which must have been well formulated and optimized in the laboratory to ascertain the right quantity needed to produce the desired inhibitory effects. This is not to under estimate the inhibitory power of bitter cola. The plant material when optimized could give better results. The most fundamental outcome established in this work is the fact the plant material tested had inhibitory properties on *Citrobacter cloaca*. All aqueous and ethanolic treatment levels are inhibitory. At higher concentration of the ethanolic extracts, zones of inhibition increased although aqueous extracts gave higher zones of inhibition in all concentrations except at 100mg that produced the same inhibition zone as ethanolic extract. As some level of microbial resistance was observed in

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Vol. 9 Issue 02
February 2022

some plates, proper formulation and processing of bitter cola may likely yield desired effects on *C. cloaca*.

Enterobacter braakii tend to be more resistant to the plant extract than the other test organism due to the low zone of inhibition observed especially under aqueous extract of bitter cola. Susceptibility improved a little with the use of ethanolic extracts possible because ethanol in itself is a disinfectant that kills microorgansms. It is therefore likely than bitter cola in itself may not be sufficient alone to kill Enterobacter *braakii*. According to Barber *et al.* (2003), microbes in the presence of an antibiotic are either killed or they survive or if they carry the resistance genes. These survivors will replicate and their progenies will quickly become the dominant type throughout the microbial population (Barber *et al.,* 2003). Comparatively, aqueous bitter cola extract tested in this work had more inhibitory effect on *Citrobacter clocae* than the ethanolic extracts but ethanolic extracts had more inhibitory effect on *Enterobacter braakii* than the aqueous extract. The later microorganism (*Enterobacter braakii*) is more resistant to bitter cola than the former (*Citrobacter clocae*). A major problem in the treatment of many ailments of bacterial origin is the occurrence of antibiotic resistance (Zella and Israel, 2013).

Results have shown that natural plant products are medicinal in nature by possessing antibacterial properties. Present report is consistent with previous investigations on the use of natural plant products to treat microbial infections (Aladesanmi *et al.,* 2007; Balogun and Owoseeni, 2013; Ikotun et al., 2017). The antibacterial efficacy of bitter cola reported in this work is in conformity with other reports where many underutilized Nigerian plants that possess useful medicinal properties should be explored (Rafia *et al.,* 2012; Nwakaeze *et al.*,2014). The presence of pathogenic bacteria capable of causing human infections has been documented in food, fruits, water, air and other media (Hassan *et al.,* 2006; Abdullahi and Abdulkareem, 2010). These opportunistic pathogens pose serious public health risks. It has been argued that antimicrobial resistance is less common with natural products compared to synthetic drugs (Zella and Israel, 2013). Moreover, natural plant products are cheaper and more accessible. Thus, when property optimized in pharmacological research, bitter cola could be explored as a cheap source of antimicrobial agent in the treatment of bacterial infections.

Table 1: Qualitative Susceptibility Test of aqueous Bitter Cola Extracts on *Citrobacter clocae*

Concentration	Plate 1	Plate 2	Plate 3	Overall Plates Result
20mg	Resistant	Resistant	Susceptible	33.3% susceptibility
40mg	Resistant	Resistant	Susceptible	33.3% susceptibility
60mg	Susceptible	Susceptible	Susceptible	100% susceptibility
80mg	Susceptible	Susceptible	Resistant	66.7% susceptibility
100mg	Resistant	Susceptible	Susceptible	66.7% susceptibility

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Vol. 9 Issue 02
February 2022

Table 2: Measurement of Zones of Inhibition of aqueous Bitter Cola Extracts on *Citrobacter clocae*

Concentration	Zones of Inhibition (mm) in Plate 1	Zones of Inhibition (mm) in Plate 2	Zones of Inhibition (mm) in Plate 3	Highest Zones of Inhibition (mm) recorded
20mg	0	0	10	10
40mg	0	0	15	15
60mg	12	7	7	12
80mg	14	10	0	14
100mg	0	10	15	15

(χ^2 @ 4df= 1.4, P=0.840, P>0.05).
Ciprofloxacin control= 20mm Zones of Inhibition
Ethanol control= 16mm Zones of Inhibition

Table 3: Qualitative Susceptibility Test of Ethanolic Bitter Cola Extracts on *Citrobacter clocae*

Concentration	Plate 1	Plate 2	Plate 3	Overall Plates Result
20mg	Resistant	Resistant	Susceptible	33.3% susceptibility
40mg	Susceptible	Susceptible	Resistant	66.7% susceptibility
60mg	Resistant	Susceptible	Resistant	33.3% susceptibility
80mg	Susceptible	Resistant	Susceptible	66.7% susceptibility
100mg	Susceptible	Susceptible	Susceptible	100% susceptibility

Table 4: Measurement of Zones of Inhibition of Ethanolic Bitter Cola Extracts on *Citrobacter clocae*

Concentration	Zones of Inhibition (mm) in Plate 1	Zones of Inhibition (mm) in Plate 2	Zones of Inhibition (mm) in Plate 3	Highest Zones of Inhibition (mm) recorded
20mg	0	0	7	7
40mg	10	5	0	10
60mg	0	6	0	6
80mg	12	0	12	12
100mg	15	14	13	15

(χ^2@ 4df = 5.4, P=0.249, P>0.05)
Ciprofloxacin control= 20mm Zones of Inhibition
Ethanol control= 16mm Zones of Inhibition

Table 5: Comparative Effects of Aqueous and Ethanolic Bitter Cola Extracts on *Citrobacter clocae*

Concentration	Qualitative Overall Plates Result (%)	Qualitative Overall Plates Result (%)	Highest Zones of Inhibition (mm) recorded	Highest Zones of Inhibition (mm) recorded
	Aqueous extracts	Ethanolic extracts	Aqueous extracts	Ethanolic extracts
20mg	33.3% susceptibility	33.3% susceptibility	10	7
40mg	33.3% susceptibility	66.7% susceptibility	15	10
60mg	100% susceptibility	33.3% susceptibility	12	6
80mg	66.7% susceptibility	66.7% susceptibility	14	12
100mg	66.7% susceptibility	100% susceptibility	15	15

T-test (aqueous.ethanolic) =1.68, P=0.145, P>0.05)
Ciprofloxacin control= 20mm Zones of Inhibition
Ethanol control= 16mm Zones of Inhibition

Table 6: Qualitative Susceptibility Test of Aqueous Bitter Cola Extracts on *Enterobacter braakii*

Concentration	Plate 1	Plate 2	Plate 3	Overall Plates Result
20mg	Resistant	Resistant	Resistant	0% susceptibility
40mg	Resistant	Resistant	Resistant	0% susceptibility
60mg	Susceptible	Resistant	Resistant	33.3% susceptibility
80mg	Susceptible	Resistant	Resistant	33.3% susceptibility
100mg	Resistant	Resistant	Resistant	0% susceptibility

Table 7: Measurement of Zones of Inhibition of Aqueous Bitter Cola Extracts on *Enterobacter braakii*

Concentration	Zones of Inhibition (mm) in Plate 1	Zones of Inhibition (mm) in Plate 2	Zones of Inhibition (mm) in Plate 3	Highest Zones of Inhibition (mm) recorded
20mg	0	0	0	0
40mg	0	0	0	0
60mg	6	0	0	6
80mg	5	0	0	5
100mg	0	0	0	0

χ^2 @4df= 16.72, P=0.002, P<0.05)
Ciprofloxacin control= 20mm Zones of Inhibition
Ethanol control= 10mm Zones of Inhibition

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Vol. 9 Issue 02
February 2022

Table 8: Qualitative Susceptibility Test of Ethanolic Bitter Cola Extracts on *Enterobacter braakii*

Concentration	Plate 1	Plate 2	Plate 3	Overall Plates Result
20mg	Resistant	Susceptible	Susceptible	66.7% susceptibility
40mg	Resistant	Resistant	Susceptible	33.3% susceptibility
60mg	Resistant	Resistant	Susceptible	33.3% susceptibility
80mg	Resistant	Resistant	Resistant	0% susceptibility
100mg	Susceptible	Susceptible	Susceptible	100% susceptibility

Table 9: Measurement of Zones of Inhibition of Ethanolic Bitter Cola Extracts on *Enterobacter braakii*

Concentration	Zones of Inhibition (mm) in Plate 1	Zones of Inhibition (mm) in Plate 2	Zones of Inhibition (mm) in Plate 3	Highest Zones of Inhibition (mm) recorded
20mg	0	10	8	10
40mg	0	0	8	8
60mg	0	0	7	7
80mg	0	0	0	0
100mg	6	7	5	7

χ^2 @4df= 8.9, P=0.063, P>0.05
Ciprofloxacin control= 20mm Zones of Inhibition
Ethanol control= 10mm Zones of Inhibition

Table 10: Comparative Effects of Aqueous and Ethanolic Bitter Cola Extracts on *Enterobacter braakii*

Concentration	Qualitative Overall Plates Result (%)	Qualitative Overall Plates Result (%)	Highest Zones of Inhibition (mm) recorded	Highest Zones of Inhibition (mm) recorded
	Aqueous extracts	**Ethanolic extracts**	**Aqueous extracts**	**Ethanolic extracts**
20mg	0% susceptibility	66.7% susceptibility	0	10
40mg	0% susceptibility	33.3% susceptibility	0	8
60mg	33.3% susceptibility	33.3% susceptibility	6	7
80mg	33.3% susceptibility	0% susceptibility	5	0
100mg	0% susceptibility	100% susceptibility	0	7

Ciprofloxacin control= 20mm Zones of Inhibition
Ethanol control= 10mm Zones of Inhibition

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Vol. 9 Issue 02
February 2022

Table 11: Comparative Zones of Inhibition of Aqueous and Ethanolic Bitter Cola Extracts on *Citrobacter clocae* and *Enterobacter braakii*

Concentration	Aqueous extracts		Ethanolic extracts	
	Citrobacter clocae	***Enterobacter braakii***	***Citrobacter clocae***	***Enterobacter braakii***
20mg	10	0	7	10
40mg	15	0	10	8
60mg	12	6	6	7
80mg	14	5	12	0
100mg	15	0	15	7
Ciprofloxacin(C1)	20	20	20	20
Ethanol (C2)	16	10	16	10

CONCLUSION

Bitter cola extracts showed inhibitory properties on the two test organisms under different extracts. *Citrobacter cloaca* was susceptible to both aqueous and ethanolic extracts of bitter cola at all levels of treatments. *Enterobacter braakii* tend to be more resistant to the plant extract than the other test organism due to the low zone of inhibition observed especially under aqueous extract of bitter cola. Comparatively, aqueous bitter cola extract had more inhibitory effect on *Citrobacter clocae* than the ethanolic extracts but ethanolic extracts had more inhibitory effect on *Enterobacter braakii* than the aqueous extract. Thus, when properly optimized in pharmacological research, bitter cola could be explored as a cheap source of antimicrobial agent in the treatment of bacterial infections.

REFERENCES

Abdullahi, I.O., Abdulkareem, S. (2010).Bacteriological quality of some ready to eat fruits as retailed and consumed in Sabon Gari, Zaria Nigeria. Bayero. Journal of Pure and Applied Science, 3: 173 175.

Adaramoye O A, Nwaneri V.O., Anyanwu K.C., Farombi E.O. and Emerole G.O. (2005). Possible anti-atherogenic effect of kolaviron(A Garcinia kola seed extract) in hypercholesterolemic rats. Clinical and Experimental Pharmacology, and Physiology. 32(1-2):40-46

Adaramoye O. A, Farombi E.O., Adeyemi E.O. and Emerole G.O. (2005). Inhibition of human low density lipoproteinoxidation by flavonoids of Garcinia kola seeds. Pakistan Journal of Medical Sciences. 21(3):331-339.

Adebukunola, O. A, Bernice O. A., Adebayo, K. A., Olayinka, O. A., Elsie, S., and Kehinde, O. (2010). Efficacy of Garcinia kola 0.5% Aqueous Eye Drops in Patients with Primary Open Angle Glaucoma or Ocular Hypertension. Middle East African Journal of Ophthalmology. 17(1): 88–93.28

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Vol. 9 Issue 02
February 2022

Adegboye, M. F., Akinpelu, D. A. and Okoh, A. I. (2008). The bioactive and phytochemical properties of Garcinia kola (Heckel) seed extract on some pathogens. African Journal of Biotechnology. 7 (21):. 3934-3938.28

Akpantah A.O., Oremosu A.A., Noronha C.C., Ekanem T.B. and Okanlawon A.O. (2005). Effects of garcinia kola seed extract on ovulation, oestrous cycle and foetal development in cyclic female sprague -dawley rats. Nigerian Journal of Physiological Sciences. 20(1 2):58-62.

Alade, A. and Ani, R. E. (2003). Protective effects of Garcinia kola seed extract against paracetamol-induced hepatotoxicity in rats. Journal of ethnopharmacology. 29(2): 207 211.

Aladesanmi AJ, Iwalewa EO, Adebajo AC, Akinkunmi EO, Taiwo BJ, Olorunmola FO and Lamikanra A. Antimicrobial and antioxidant activi-ties of some Nigerian medicinal plants. African Journal of Traditional,Complementary and Alternative Medicines, 4(2), 2007, 173-184.

Antia B.S., Pansanit A., Ekpa O.D., Ekpe U.J., Mahidol C. and Kittakoop P. (2010). Alpha glucosidase inhibitory, aromatase inhibitory and antiplasmodial activities of a biflavonoid GB1 from Garcinia kola stem bark. Planta Medica. 76(3):276-277.

Balogun OS and Owoseeni AA. Phytochemical and antimicrobial investigation of Dissotis rotundifolia RTriana. (2013). Natural Product, 9: 77-80

Barber, D.A., Miller, G.Y., McNamara, P.E. (2003). Models of antimicrobial resistance and foodborne illness: examining assumptions and practical applications. Journal of Food Protocols, 66(4):700–709

Carbonne, A., Arnaud, I., Maugat, S., Marty, N., Dumartin, C. and Bertrand, X. (2013). National multidrug-resistant bacteria (MDRB) surveillance in France through the RAISIN network: a 9 year experience. Journal of Antimicrobial Chemotherapy. 68:954–959.

Charity, U. O., Uwaifoh, A., Charlse, I. I., Williams, A. A. and Kerry, E. A. (2012). The effect of chronic ingestion of crude Garcinia kola on the histology of the liver. Pelagia Research Library European Journal of Experimental Biology. 2 (2):404-409.30

Chevalier, J., Mulfinger, C., Garnotel, E., Nicolas, P., Davin-Régli, A., and Pagès, J. M. (2008). Identification and evolution of drug efflux pump in clinical Enterobacter aerogenes strains isolated in 1995 and 2003. PLoS ONE 3: 32-43.

Christinah T.S. and Roland N.N. (2012). Identification and Antibacterial Evaluation of Bioactive Compounds from Garcinia kola (Heckel) Seeds. Molecules.; 17: 6569-6584.

Collise N., Anthony, J. A., Anna, M. C. and Roland, N. N. (2011). Crude Ethanolic Extracts of Garcinia kola Seeds Heckel (Guttiferae) Prolong the Lag Phase of Helicobacter pylori: Inhibitory and Bactericidal Potential. Journal of Medicinal Food. 14(7-8): 822-827.30

Dauda Z. (2014). Evaluation of hepatoprotective effectof aqueous extract of bitter kola (Garcinia kola) seeds on carbon tetrachloride (ccl4) induced liver damage in adult wistar rat. A thesis submitted to the school of post graduate studies, ahmadu bello university, zaria, in partial fulfillment of the requirements for the award of master of science (m.sc) degree in human anatomy department of human anatomy faculty of medicine AhmaduB University, Zaria, Nigeria, 2014.

Diene, S.M., Merhej, V., Henry, M., El Filali, A., Roux, V., Robert, C. (2013). The rhizome of the multidrug-resistant Enterobacter aerogenes genome reveals how new “killer bugs” are created because of a sympatric lifestyle. Molecular Biology and Evolution. 30:369 -383.

Esimone C.O., Adikwu M.U., Nworu C.S., Okoye F.B.C. and Odimegwu D.C. (2007). Adaptogenic potentials of Camellia sinensis leaves, Garcinia kola and Kola nitida seeds. Science Research Essays. 2:232-237.

Esimone, C.O., Nwafor, S.V., Okoli, C.O., Chah, K.F., Uzuegbu, D.B., Chibundu, C., Eche, M.A., and Adikwu, M.U. (2002). In Vivo Evaluation of Interaction Between Aqueous Seed Extract of Garcinia kola Heckel and Ciprofloxacin Hydrochloride. American Journal of Therapeutics. 9 (4):275-280

Gabriel, F.I. and Emmanuel, O.O. (2011). Pharmacological evaluation of Garcinia kola nut for antitrichomonal activity. International Journal of Pharmacological and Biological Sciences. 2(2): 264-269.

Gayet, S., Chollet, R., Molle, G., Pagès, J. M., and Chevalier, J. (2003). Modification of outer membrane protein profile and evidence suggesting an active drug pump in Enterobacter aerogenes clinical strains. Antimicrobial Agents for Chemotherapy. 47:1555–1559.

Greenwood, D. (2007). Medical Microbiology: A Guide to Microbial Infections: Pathogenesis, Immunity, Laboratory Diagnosis, and Control. Churchill Livingstone/Elsevier, New York.32

Hassan, A., Utku, O., Koray, K. (2006). Determination of total aerobic and indication. Bacteria on some raw eaten vegetables and fruits from wholesalers in Ankara, turkey. International Journal of Hygiene and Environmental Health, 209: 197 201.

Ibikunle GF, Ogbadoyi EO (2011). Pharmacological evaluation of Garcinia kola nuts for anti trichomonal activity. International Journal of Pharmacology and Biological Sciences 2(2):264-269.

Ikotun AA, Olalere CA, Adekunle DO and Dawodu MO. Phytochemistry and Antibacterial Studies of African Black Soap and its Modified Samples (2017 a) J Chem Pharmaceut Res 9: 354-359.

Iwu, M. (2013). Handbook of African medicinal plants. CRC Press, Boca Raton, FL. pp12: 32 - 38

Iwu, M.M., Igboko, O.A. Okunji C.O. and Tempesta M.S. (2011). Antidiabetic and aldose reductase activities of Biflavanones of Garcinia kola. Journal of Pharmaceutical Pharmacology. 42:290-292.33

Iwuji, T.C. and Herbert, U. (2012). Semen characteristics and libido of rabbit bucks fed diets containing Garcinia kola seed meal. Rabbit Genetics. 2(1):10-14.

Izdebski, R., Baraniak, A., Herda, M., Fiett, J., Bonten, M. J., Carmeli, Y. (2014). MLST reveals potentially high-risk international clones of Enterobacter cloacae. Journal of Antimicrobial Chemotherapy. 70:48–56. 33

Jarlier, V., and INVS. (2014). Surveillance of Multidrug Resistant Bacteria in French Healthcare Facilities BMR-Raisin Network Données 2012. Saint-Maurice:Institut de Veille Sanitaire.

Juliana, A., Moctar, S. and Christophe, K. (2006). Garcinia kola Heckel. Forest & Landscape Denmark.33

Kakjing, D. F., Mary, O. U. and Nkoli, L. N. (2014). Anti-pyretic Activity of Garcinia kola33Seed Extract. European Journal of Medicinal Plants .4(5). 511-52133

Konziase B. (2015). Protective activity of biflavanones from Garcinia kola against Plasmodium infection. Journal of Ethnopharmacology. 172:214-218

Lacmata S.T., Kuete V., Dzoyem J.P., Tankeo S.B., Teke G.N., Kuiate J.R. and Pages J.M. (2012). Antibacterial activities of selected Cameroonian plants and their synergistic

effects with antibiotics against bacteria expressing MDR phenotypes. Evidence Based Complementary and Alternative Medicine. doi: 10.1155/2012/623723

Landberg R., Sun Q. and Rimm E.B. (2011). Selected dietary flavonoids are associated with markers of inflammation and endothelial dysfunction in US Women. Journal of Nutrition. 141:618-625.

Langley, J. M., Hanakowski, M., and Leblanc, J. C. (2001). Unique epidemiology of nosocomial urinary tract infection in children. American Journal of Infections Control 29: 94–98.

Lavigne, J.P., Sotto, A., Nicolas-Chanoine, M.H., Bouziges, N., Bourg, G., Davin-Regli, A. (2012). Membrane permeability, a pivotal function involved in antibiotic resistance and virulence in Enterobacter aerogenes clinical isolates. Clinical Microbiological Infections. 18:539–545

Mathew, O.W. and Blessing C.D. (2007). Hepatoprotective effects of Garcinia kola seed against hepatotoxicity induced by carbon tetrachloride in rats. BIOKEMISTRI. 19(1):17-21.34

Mazi E.A., Okoronkwo K.A. and Ibe U.K. (2013). Physico-Chemical and Nutritive Properties of Bitter Kola (Garcinia Kola). Journal of Nutrition and Food Sciences 3:218-224.

McKeel, R., Douris, N., Foley, P.L. and Feldman, S.H., (2002). Comparison of an espB gene fecal polymerase chain reaction assay with bacteriologic isolation for detection of Citrobacter rodentium infection in mice. Complimentary Medicine. 52: 439–444.34

Mesembe, O.E., Asuquo, O.R., Fischer, V.A., Udoaffah, G.U., Mfem, C.C and Kalu, N.N. Influence of Long-term Ingestion of Garcinia Kola Seed Diet on Sperm Count, Sperm Motility, and Fertility in the Wistar Rat. Journal of Health, Medicine and Nursing. 1:20 22.

Mezzatesta, M.L., Gona, F., and Stefani, S. (2012). Enterobacter cloacae complex: clinical impact and emerging antibiotic resistance. Future of Microbiology. 7:887–90

Nwakaeze AE, Iroha IR, Ejikeugwu PC, Afiukwa FN, Udu-Ibiam OE, Oji AE, Agumah BN and Eze EA. Evaluation of antibacterial activities of some Nigerian medicinal plants against some resistant bacterial pathogens. African Journal of Microbiology Research, 8(18), 2014, 1832-1836.

Nwangwa, E.K. (2012). Effects of Garcinia kola on the Lipid Profile of Alloxan-Induced Diabetic Wistar Rats. British Journal of Pharmacology and Toxicology. 3(2):39-42.3535

Nwokochaa, CR., Owua, DU., Ufearob, CS. and Iwualac, M.O.E. (2011). Comparative study on the efficacy of Garcinia kola in reducing some heavy metal accumulation in liver of Wistar rats. Journal of Ethnopharmacology. 135 (2): Pages 488–491.

Obi A.U. and Nwoha P.U. (2014). Effects of Kolaviron, the Major Constituent of Garcinia kola, on the Histology of the Hypothalamus, Pituitary, and Testes Using Adult Male Wistar Rats as a Model Organism. Forensic Medicine and Anatomy Research. 2:80-87.

Odebunmi E.O., Oluwanili O.O., Awolola G.V. and Adediji O.D. (2009). Proximate and nutritional composition of Kola nut (Cola nitida), bitter kola (Garcinia kola), and Alligator pepper (Afromomum melegueta). African Journal of Biotechnology. 8(2):308 - 310

Ofokansi, KC., Mbanefo, AN., Ofokansi, MN and Esimone, CO. (2008). Antibacterial Interaction of Crude Methanol Extract of Garcinia kola Seed with Gatifloxacin. Tropical Journal of Pharmaceutical Research; 7(4): pp1159-1165.

Okoko T., Awhin T. and Ejiro P. (2007). Garcinia kola extract reduced Cisplatin-induced dysfunction in rats. African Journal of Biochemistry Research. 1(6):124-126.

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Vol. 9 Issue 02
February 2022

Okoko, T. and Ndoni, S. (2009). The effect of Garcinia kola extract on lipopolysaccharide induced tissue damage in rats. Tropical Journal of Pharmaceutical Research; 8 (1):27 31.36

Olaleye S.B., Farombi E.O., Adewoye E.A., Owoyele B.V., Onasanwo S.A. and Elegbe R.A. (2000). Analgesic and anti-inflammatory effects of Kolaviron (a garcinia kola seed extract). African Journal of Biomedical Research. 3:171-174

Olatunde E., Farombi A., Akanni O.O. and Emerole G.O. (2008). Antioxidant and Scavenging Activities of Flavonoid Extract (Kolaviron) of Garcinia kola Seeds. Pharmaceutical Biology. 40(2):107-116.

Oloyede O.I. and Afolabi A.M. (2012). Antioxidant potential of Garcinia kola (leaf). Academic Research International. 2(2):49-54.

Oluwatosin A., Tolulope A., Ayokulehin K., Okorie P., Aderemi K., Falade C. and Olusegun A. (2014). Antimalarial potential of kolaviron, a biflavonoid from Garcinia kola seed against Plasmodium berghei infectionin Swiss albino mice. Asian Pacific Journal of Tropical Medicine. 2014:97-104.

Onasanwo, S. A., Singh, N., Olaleye, S. B. and Palit, G. (2011). Anti-ulcerogenic and proton pump (H+, K+ ATPase) inhibitory activity of 37Kolaviron from Garcinia kola Heckel in rodents. Indian Journal of Experimental Biology. 49(6): 461-468.

Otor, J. U., Abdulkadir, U. and Abu, M. A. (2001). Some biological activities of Garcinia kola in growing rats. Veterinary Archive. 71 (5): 287-297.

Penduka, D., Okoh, OO. and Okoh, AI. (2011). In Vitro Antagonistic Characteristics of Crude Aqueous and Methanolic Extracts of Garcinia kola (Heckel) Seeds against Some Vibrio Bacteria. Molecules. 16(4):2754-2765.

Pestourie, N., Garnier, F., Barraud, O., Bedu, A., Ploy, M.C., and Mounier, M. (2014). Outbreak of AmpCβ-lactamase-hyper-producing Enterobacter cloacae in a neonatal intensive care unit in a French teaching hospital. American Journal of Infections Control. 42:456–458.

Potron, A., Poirel, L., Rondinaud, E., and Nordmann, P. (2013). Intercontinental spread of OXA 48 β-lactamase-producing Enterobacteriaceae over an 11-year period, 2001 to 2011. European Surveillance. 18:205-249.

Rafia, A, Hussein, El-Saltani, Nabeel, S, Asma, Al-Najjar and Fatma,H. Antioxdant and antibacterial properties of five medicinal Libyan plants extract. Natural Science, 4(5), 2012, 324-335

Seanego C.T. and Ndip R.N. (2012). Identification and antibacterial evaluation of bioactive compounds from Garcinia kola (Heckel) seeds. Molecules 17(6):6569-6584.

Tebekeme, O. (2009). In vitro antioxidant and free radical scavenging activities of Garcinia kola seeds. Food and Chemical Toxicology. 47(10):2620–2623.

Terashima K., Takaya D. and Niwa M. (2002). Powerful antioxidative agents based on garcinoic acid from Garcinia kola. Bioorganic Medicinal Chemistry. 10(5):1619-1625.

Udenze, E.C.C. Braide, V.B. Okwesilieze, C.N. and Akuodor, G.C. (2012). Pharmacological Effects of Garcinia kola Seed Powder on Blood Sugar, Lipid Profile and Atherogenic Index of Alloxan-induced Diabetes in Rats. Pharmacologia. 3(12):693-699.3838

Unigwe, C.R. and Nwakpu, P.E. (2009). Effect of ingestion of Garcinia kola seed on erythrocytes in rabbits. Continental Journal of Veterinary Sciences. 3: 7 – 10.

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Vol. 9 Issue 02
February 2022

Wink M., Schmeller T. and Laty-Bruning B. (1998). Modes of action of allele-chemical alkaloids: interaction with neuroreceptor, DNA and other molecular targets. Journal of Chemical Ecology. 24:1881-1937.

Zella, G.C. and Israel, E.J. (2013). Chronic Diarrhoea in Children. Paediatrics in Review, 33 (5): 207-304