

**ANTI-BACTERIAL ACTIVITY OF VARIOUS BLENDS OF AQUEOUS AND ETHANOL  
EXTRACTS OF GARLIC AND BITTER COLA**

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**ABSTRACT**

*Diameter of inhibitory zone on Staphylococcus aureus, Escherichia coli and Streptococcus pyogenes were determined by evaluating Ethanol and water extract of bitter kola (Garcinia kola) and garlic (Allium sativum) at concentrations of sample A: (BC 100:0 G), B: (BC 80:20 G), C: (BC 60:40 G), D: (BC 40:60), E: (BC 20:80 G), F: (BC 0:100 G) and all concentrations were treated with 150ml ethanol and water extracts. For this experiment, the bitter kola and garlic were dried and milled and biotic extraction was done at different concentrations or ratios. The results showed that the diameter of inhibition for E.coli and Staphylococcus aureus on ethanol extracts values ranged from (8.20mm -19.22mm) and (8.22mm – 20.16mm) respectively with sample D (40g of Bitter kola + 60g of garlic) has the highest diameter of inhibition with a value of 1.92mm for E.coli and 2.07mm for staphylococcus aureus making it a good antimicrobial agent. While for Streptococcus aureus, the extracted ethanol solvent of sample C (60g of bitter kola + 40g of garlic) has the higher diameter of inhibition value of 1.15mm. Water extract values of diameter of Inhibitory zone were generally lower than the ethanol extract values for all organisms listed. This implies that water extract exhibited lower capacity of microbial inhibition to E.coli, streptococcus and staphylococcus aureus under the condition of study.*

**Keywords:** Garlic, Garcinia kola, Diameter of inhibitory zone, E.coli, Streptococcus, Staphylococcus aureus, Ethanol extract

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**INTRODUCTION**

The use of plant extracts in the treatment of diseases have become of interest over the years due to the fact that microorganisms are developing resistance to many drugs and as such created situation where some of the common and less expensive antimicrobial agents are losing

effectiveness. There is an urgent need to find an alternative to chemotherapeutic drugs in disease treatment, particularly those of plants origin which are easily available and have considerably less side effects (khuibe and Sati, 2009). *Garcinia kola* is commonly referred to in Nigeria as “Bitter kola” due to its characteristic bitter taste. The plant is grown in the humid regions of Western and Central Africa (Penduka *et al.* 2011). The kernel contains a wide range of useful phytochemicals such as high contents of tannins and flavonoids. Various researches have reported the presence of a biflavonoid known as kolaviron complex. Kolaviron has been shown to demonstrate functional properties in neuroprotection, antimicrobial, and many other functions favorable to human health (Usunomena, 2012). Also, the extracts of the *Garcinia* seeds are reported by Tchimine *et al.* (2016) and Ajayi *et al.* (2011) to have anti-inflammatory effects. In addition, kolaviron possesses anti-malarial and wound healing properties (Nwachujor *et al.*, 2015). Therapeutic potential of kolaviron was shown in treatment of benign prostatic hyperplasia (Kalu *et al.*, 2016). Nwaokorie *et al.* (2010) reported the effect of the extracts on *Fusobacterium nucleatum* while the methanolic and aqueous extracts of the kola seeds were evaluated by Penduka *et al.* (2011) on *Vibrio* isolates and reported that both extracts inhibited the activities of the tested organisms.

Garlic (*Allium sativum* L.) is under family Liliacea. It is an erect annual herb with superficial adventitious roots, bulbs composed of a disk like stem (Abebe, 2013). Garlic has an unusually high concentration of sulfur-containing compounds (1-3%) and its therapeutic properties are largely due to one particular class of sulfur-containing compounds, the thiosulfinates (Lawson, 2006). The thiosulfinate structure appears to be essential for the bactericidal, antifungal and antiprotozoal properties of garlic, likely reacting with SH-containing enzymes of these pathogens (Reuter *et al.*, 2006)

Garlic also has anti-bacterial activity against bacterial pathogens including Gram positive and Gram negative bacteria (Eja *et al.*, 2007; Durairaj *et al.*, 2009). A bioactive compound in garlic that has anti-bacterial activity is allicin, which is a volatile compound containing sulfur (Harris 2001). Other bioactive compounds such as diallyl disulphide, and diallyl trisulphide, have also been shown to have antibacterial activity as reported by Avato *et al.* (2000), Tsao and Yin (2001).

Antimicrobial activity of garlic extracts are affected by extraction solvent and method of extraction, with ethanol extracts being more effective than aqueous extract (Akullo et al., 2022). Garlic extracts exhibit broad spectrum antibiotics against both gram positive and gram negative bacteria and fungi. The anti-bacterial and antimycotic activity of garlic increases with increased concentration of the garlic extract. Garlic extract enhances the antibacterial activity and protects an individual from bacterial invasion (Fufa, 2019)

There are several reports of antibacterial activity of aqueous garlic extract (AGE) against a variety of bacteria. In vitro assay with AGE (10%) showed complete inhibition of *Bacillus cereus* and the activity varies upon the storage conditions and heat treatment of the aqueous extract. AGE exhibited in vitro antibacterial activity against various pathogenic bacteria including *Shigella* and *Salmonella* species and enterotoxigenic *E. coli* (Bhatwalkar et al, 2021). The principal phytochemicals that exhibit antibacterial activity are oil-soluble organosulfur compounds that include allicin and allyl sulfides and they exhibit such antibacterial properties as bactericidal, antibiofilm, antitoxin and anti-quorum sensing activities. The reactive organosulfur compounds form disulfide bonds with free sulfhydryl groups of enzymes and compromise the integrity of bacterial membrane.

Currently, there is growing interest in exploiting plants for medicinal purposes especially in Africa and this may stem from the fact that microorganisms are developing resistance to many drugs and this has created a situation where some of the common and less expensive conventional antimicrobial agents are losing their efficacy (Montefiore et al., 1989). The objective of this study is to assess the inhibitory activity aqueous and ethanol extracts of bitter kola (*Garcinia kola*) and garlic (*Allium sativum*) on microbial pathogens and to determine the extract concentration with the highest diameter of inhibitory zone.

## **MATERIALS AND METHODS:**

### **Source of Experimental Materials**

The bitter kola (*Garcinia kola*) and garlic (*Allium sativum*) seeds were purchased from oil mill Market, Port Harcourt. *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus* were cultured

in the Microbiology and Fermentation Laboratory, Department of Food Science and Technology, Rivers State University, Port Harcourt.

### **Experimental Design**

Completely randomized design (CRD) consisting of 6 treatments and control and each treatment replicated 3 times was used for the study for each plant (bitter kola (*Garcinia kola*) and garlic (*Allium sativum*)) against each pathogen (*Escherichia coli*, *Staphylococcus aureus* and *Streptococcus sp.*).

### **Preparation of Extract:**

Bitter cola samples were peeled and cut into smaller pieces of 1cm in diameter. The cut bits were dried in a hot air oven with temperature of 55<sup>0</sup>C for 8hr. This was then milled to powder and stored for use. The garlic samples were sorted, peeled and milled in a blender and the various ratios of garlic and bitter cola (weight/weight) as laid out in the experiment design was weighed in duplicate samples into a conical flask and 200ml of ethanol (distilled water for aqueous extract) added and allowed to stand for 24 hr at room temperature with intermittent shaking. These were filtered with Whatman filter paper no 1 and the filtrate concentrated in a rotary evaporator at 40<sup>0</sup>C and stored in a sterilized bottle for use.

### **Preparation and Identification of Isolates:**

Bacterial colony consisting of *E.coli*, *Staphylococcus aureus* and *Streptococcus* were selected from their cultured plate and sub cultured in nutrient agar by the streaking method and incubated at 37<sup>0</sup>C for 24hr. Size of colony, Shape, pigmentation, texture, opacity and consistency were used in the identification of isolates. This is used to classify bacteria organisms to two groups (Gram positive and Gram-negative bacteria). This technique differentiates the bacteria base on their coloring properties Further confirmatory tests were conducted by standard procedures to ascertain biochemical characteristics through oxidase test, mortality test, catalase test, indole and coagulase test.

Determination of diameter of diameter of inhibitory zone was carried in accordance with the Agar diffusion method described by Olajuyigbe *et.al*, (2020). The different extract preparations were used on test organisms to determine the diameter of inhibitory zone.

### **Data Collection**

Data was collected on the diameter of inhibitory zone for *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus sp.*

### **Statistical Analysis**

Data collected was subjected to one - way Analysis of variance (ANOVA) to test for significant differences ( $p < 0.05$ ) among treatment using Genstat 14.0 software.

## **RESULTS**

The data presented in Table 2 shows the result of the biochemical test carried out to confirm the identity of the organism used in the experiment. Gram staining was positive for *Staphylococcus aureus* but negative for *E.coli* and streptococcus. Oxidase test was negative for both *Staphylococcus aureus* and *E.coli* while streptococcus was positive, motility test showed positive for *E.coli* and negative for *Staphylococcus aureus* and streptococcus, catalyse test gave positive result for both *Staphylococcus aureus*, and *E.coli* while streptococcus was negative, indole test indicated the *E.coli* was positive while *Staphylococcus aureus* and streptococcus was negative and coagulase test was positive for *Staphylococcus aureus* and negative for *E.coli* and streptococcus

Figure1: Shows the result of the diameter of the inhibitory zone of ethanol extract on *E. coli* with values ranging from (8.20mm – 19.22mm). The result showed that Sample D (40g bitter kola and 60g garlic) had a value of 19.22mm, indicating that extracts from sample D exhibited a higher diameter of inhibitory zone for *E.coli* than other extract concentration. Results indicate that increasing the concentration of garlic beyond 40g led to a reduction in the diameter of inhibition and similarly values above 60g of bitter kola did not improve the performance of the extracts as well as values below 40g of garlic. It would appear that Sample D is the optimal threshold value for microbial inhibition of *E.coli*.

Diameter of inhibition for *Streptococcus* showed a range of values from 0.65mm-11.05mm, with extracted solvent of sample D (40g of bitter kola and 60g of garlic) been significantly higher than all other extracts used in the test against *Streptococcus Spp.* This shows that Sample A (100g of bitter kola) had the least value of 0.65mm and consequently lowest diameter of inhibition.

Diameter of inhibition for *Staphylococcus* had a range of values from (8.22mm – 20.16mm). The highest diameter of inhibitory zone of 20.16mm was shown by Sample D (40g of bitter kola and 60g of garlic) meaning that Sample D had the threshold concentration of microbial inhibition. Concentration above or below Sample D would lead to decrease in the diameter of inhibition. Similar results have been reported by Enejiyon et.al, (2020) , who reported the antibacterial activity of garlic ethanol extracts on gram positive and gram negative bacteria.

Figure 2: Shows the results of water extract values of diameter of inhibitory zone which are generally lower than the ethanol extract values for all the organisms listed. This implies that water extract exhibited lower capacity of microbial inhibition to *E.coli*, *Streptococcus* and *Staphylococcus aureus* under the conditions of study. However, Sample C (60g of bitter kola and 40g of garlic) exhibited a significantly higher diameter of inhibition of 0.38mm for *Staphylococcus aureus*. *Streptococcus* organism showed resistance to water extracts of all the treatments used in the study. This indicates that water extracts will not be effective in inhibiting microbial growth of *streptococcus*.

Table 3: shows antibiotics disc gram negative sensitivity zone of inhibition. From the table, Ciprofloxacin had the highest sensitivity followed by Peflozacin while the organisms were resistant to septrin, Augumentin, Tarivid, and Streptomycin.

Table 4: shows antibiotics disc gram positive sensitivity zone of inhibition. From the table, Ciprofloxacin, Septrin, Peflozacin and Streptomycin had the highest sensitivity followed by Erythromycin, Rifanpicin while the organisms were resistant to Chloramphenicol, Sparfloxacin, Amozacillin, Augumentin, Gentamycin, Tarivid, and Ampiclox.

## **DISCUSSION**

The diameter of inhibitory zone values *E.coli* ranged from 8.22mm – 19.22mm with Sample D (40g of bitter kola and 60g od garlic) having the highest diameter of inhibitory zone of 19.22mm which is significantly higher than other treatments for ethanol extracts. This result collaborates with the findings of (Sharma *et al.*, 2007) who reported that garlic (*Allium sativum* Linn.) possesses a broad spectrum antibacterial activity including *E.coli*. This implies that the extracted solvent has the potential of being used as an antimicrobial agent.

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The result revealed that Sample D (40g of bitter kola and 60g of garlic) had the potential for microbial inhibition for *E.coli* and also any extracts composition above or below 40g bitter kola and 60g garlic would reduce the diameter of inhibition for *E.coli*. Findings indicated that Sample A( 100g bitter cola ) was significantly lower than all other treatments. This implies that bitter kola only will not be a good antimicrobial inhibitor for *Streptococcus*. However, a combination of bitter kola (60g) and garlic (40g) which is Sample C was significantly higher than all other treatment of ethanol for *Streptococcus*.

The highest diameter of inhibitory zone of 20.16mm was shown by Sample D (40g of bitter kola and 60g of garlic) for *Staphylococcus aureus*. This implies that Sample D has the optimal threshold concentration of inhibition and this collaborates with the study by Indabawa and Arzai (2011), who reported that ethanol and water extract of bitter kola showed activity against *Staphylococcus aureus* and other organisms. According to results of Adegboye *et al.* (2008) the ethanol extract of bitter kola exhibited significant inhibitory action against eleven out of fifteen bacterial isolates (*Bacillus*, *Clostridium*, *Corynebacterium*, *Escherichia*, *Klebsiella*, *Micrococcus*, *Pseudomonas*, *Staphylococcus*).

Water extract values of diameter of inhibitory zone were generally lower than the ethanol extract values for the organisms listed. This implies that water extract exhibited lower capacity of microbial inhibition to *E.coli*, *Streptococcus* and *Staphylococcus aureus* under the conditions of study. However, Sample C (60g of bitter kola and 40g garlic) exhibited a significantly higher diameter of inhibitory zone of 0.38mm for *Staphylococcus aureus* while *Streptococcus* showed resistance to water extracts of all the treatments used in the study. This indicates that water extracts will not be effective in inhibiting microbial growth of *Streptococcus*.

## **CONCLUSION**

Ethanol treatment extract of Sample D (garlic 40g: 60g bitter cola) resulted in significantly higher value of diameter of inhibitory zone for all the micro-organisms tested viz *E.coli*, *Streptococcus* Spp and *Staphylococcus aureus* respectively. Blends of garlic and bitter cola reveal potential anti-microbial activity than either garlic or bitter used separately.

**CONFLICT OF INTEREST:** None

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**APPENDICES**

**Table 1:** Treatment formulation for Ethanol and water extract

<b>SAMPLE</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
Bitter kola (g)	100	80	60	40	20	-
Garlic (g)	-	20	40	60	80	100
Volume of solvent 200ml						

Table 2: Biochemical Test

<b>Chemical Test</b>	<b><i>E.coli</i></b>	<b><i>Staph .aureus</i></b>	<b><i>Strep</i></b>
Gram staining	-	+	+
Oxidase	-	-	+
Motility	+	-	-
Catalyse	+	+	-
Indole	+	-	-
Coagulase	-	+	-

Key: + = positive, - = negative

Table 3:Antibiotics disc gram negative sensitivity zone of inhibition (mm)

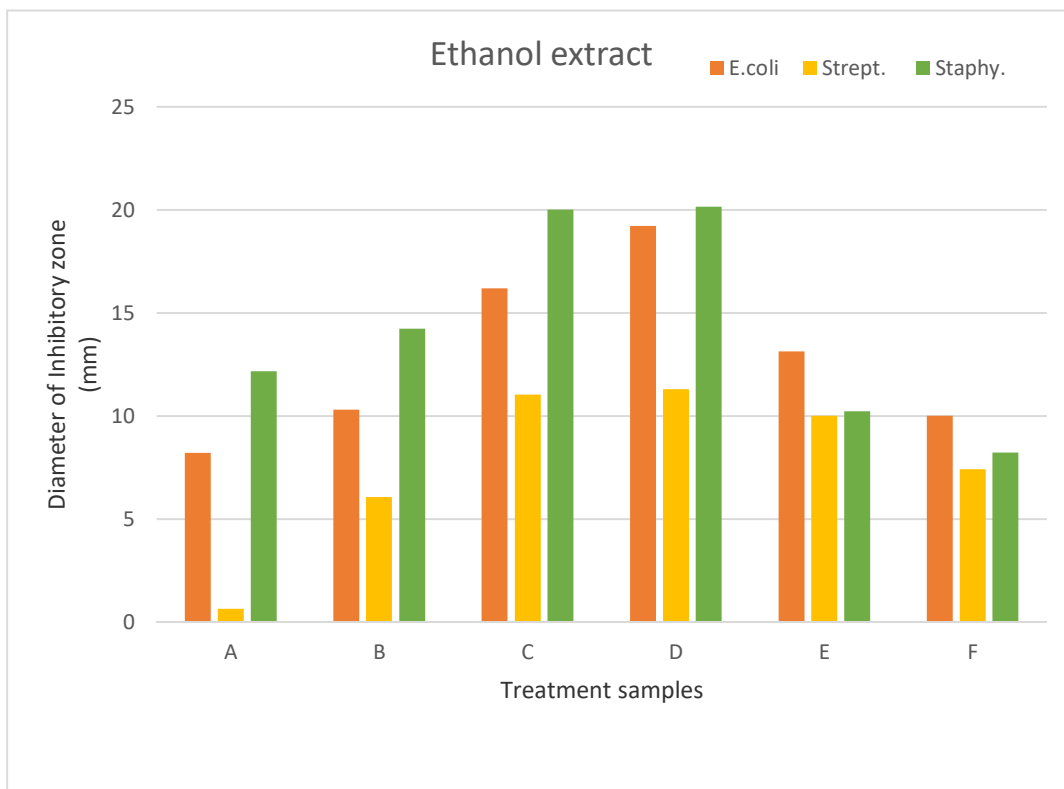
<b>Antibiotics</b>	<b>Organisms (<i>E.coli</i>)</b>	<b>Range</b>
Seprin	R	-
Chloramphenicol	S	+
Sparfloxacin	S	+
Ciproflozacin	S	+++
Amozacillin	S	+
Augumentin	S	-
Gentamycin	S	+
Peflozacin	S	++
Tarivid	R	-
Streptomycin	R	-

**Key:** R- resistance, S- sensitive. +++ highly sensitive, ++ moderately sensitive, + low sensitive – resistant

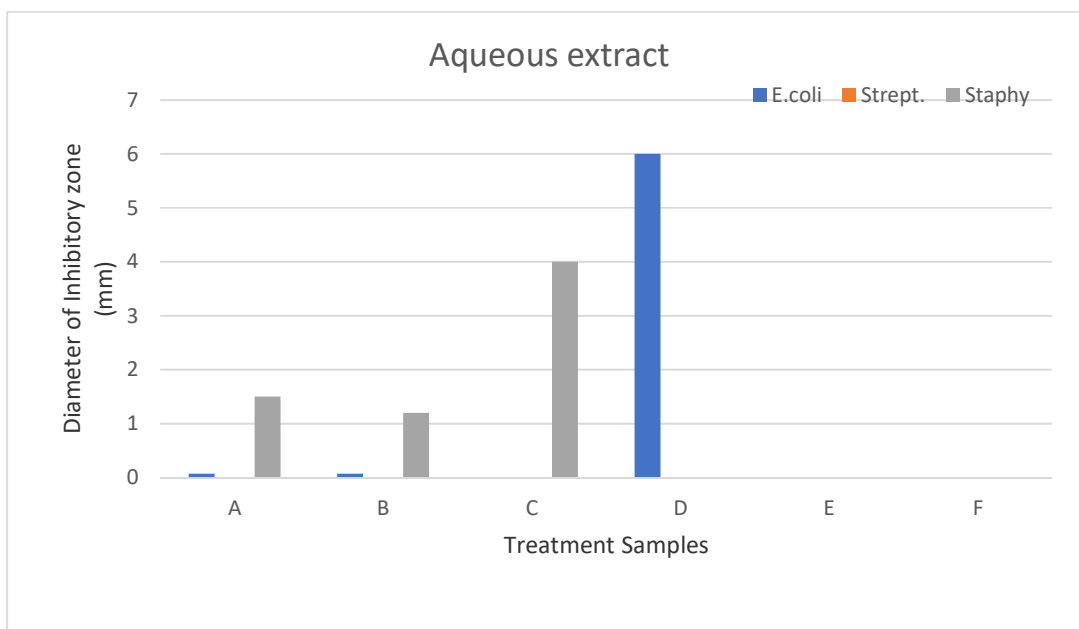
**Table 4: Antibiotics disc gram positive sensitivity zone of inhibition (mm)**

Antibiotics	Organisms ( <i>staph</i> )	Range
Septin	S	+++
Chloramphenicol	R	-
Sparfloxacin	R	-
Ciproflozacin	S	+++
Amozacillin	R	-
Augumentin	R	-
Gentamycin	R	-
Peflozacin	S	+++
Tarivid	R	-
Streptomycin	S	+++
Erythromycin	S	+
Rifampicin	S	+
Ampiclox	R	-

**Key:** R- resistance, S- sensitive. +++ highly sensitive, ++ moderately sensitive, + low sensitive – resistant



**Figure 1: showing the diameter of inhibitory zone for ethanol extract**



**Figure 2: showing the diameter of inhibitory zone for water extract**