WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 7, 1-17. Research Article ISSN 2277– 7105

IN-VITRO INTERACTION OF ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF *GARCINIA KOLA* WITH AZITHROMYCIN

Anie Clement Oliseloke[1]*, Egbon Olukayode T.[2] and Obidike Judith Chisom[1]

[1]Faculty of Pharmacy, Delta State University, Nigeria.

[2]Faculty of Pharmacy, Federal University, Ekiti, Nigeria.

Article Received on
04 April 2022,

Revised on 25 April 2022,
Accepted on 15 May 2022

DOI: 10.20959/wjpr20227-24170

***Corresponding Author**
Anie Clement Oliseloke
Faculty of Pharmacy, Delta State University, Nigeria.

ABSTRACT

Objectives: This study was conducted to evaluate the antibacterial interaction of Azithromycin with crude methanol extract of *Garcinia Kola*, the concomitant use of *Garcinia kola* seed extract and Azithromycin and the interactions that may possibly occur due to the simultaneous use of these two agents. **Method**: The plant material was gotten, peeled, washed, dried, ground and then extracted using cold maceration method with 80% methanol. Qualitative analysis was carried out, revealing the phytochemical present in the methanol extract of *Garcinia kola* was done, Antibacterial activity of the methanol extract of *Garcinia kola* at various concentrations was obtained using agar well diffusion method with Azithromycin as a positive control, and methanol as a negative control. Minimum inhibitory concentration of *Garcinia kola* and Azithromycin were evaluated using the above test organisms. The synergistic effect of azithromycin and the methanol extract of *Garcinia kola* seed was carried out. The determination of the combined effect of methanol extract and Azithromycin against *Pseudomonas aureginosa*, using the checker board method was obtained. **Results**: The presence of saponin, alkaloid, tannins, flavonoids, terpenoids and steroids was shown due to the phytochemical screening carried out. The antimicrobial evaluation showed zones of inhibition against the four test organism (*E.coli, S.aureus, Strep. pyogenes* and *P.aeruginosa*) were 3.5mm to 15mm ranging from the lowest to the highest for the various concentration for *Garcinia kola* extract. 14.5 to 33(mm) was the range obtained for the zone of inhibition for the positive Azithromycin. The MIC(Minimum Inhibitory Concentration (mg/ml) for the various micro organism which are *S.aureus, E.coli, P.aeruginosa* and *S.pyogenes* were at a concentration of 2.3437, 300, 2.3437

and 150 respectively for the methanol extract of *Garcinia kola* and 2.5, 10, 2.5 for *S.aureus*, *P.aeruginosa*, *S.pyogenes* respectively for Azithromycin. *E.coli* does not have an MIC. The synergistic effect of the methanol extract of *Garcinia kola* and Azithromycin have higher zone of inhibition against *Staphylococcus aureus* and *Escherichia coli* at the following concentration (19mm and 29mm) respectively, unlike *Pseudomonas aeruginosa* and *Streptococcus pyogenes* that showed antagonism when the extract of Azithromycin *and Garcinia kola* were combined. Using the Checker board method, the combined effect of the extract and Azithromycin against *Pseudomonas aeruginosa*, synergistic effect was seen at AZI/GKS ratios of 9:1, 8:2, 7:3, 6:4 and 5:5, indifference was seen at ratio 4:6 while antagonism was recorded at ratio 3:7, 2:8 and 1:9. **Conclusion**: From the study carried out, it can be said that *Garcinia kola* possesses antibacterial activity and the combined effect of the interaction between Azithromycin *and Garcinia kola* seed extract was synergistic against *Escherichia coli*.and *Staphylococcus aureus.*

INTRODUCTION

Plants produce many organic compounds which have value in the treatment of various diseases (Anie etal., 2011). Orthodox drugs or medicine on the other hand, are usually chemical pure substances which when they are administered into the body exert pharmacological effects that could therefore lead to alleviation of the disease or help in the diagnosis or prevention of the disorder. Many modern orthodox drugs have herbal medicine origin, but the main difference between the two is that; the herbal drugs contain a good number of compounds, instead of a single pharmacologically active substance, for this reason constituents of both herbal and orthodox medicines might act on one another to enhance or oppose their effect (Houghton 2009). Concurrent use of herbal and orthodox medicine causes interaction between these two forms of medicine which can lead to undesirable pharmacokinetic and pharmacodynamics effect. Some medicinal plants do not only possess antibacterial activities but also antimalarial properties (Akpo etal., 2020) This in the recent past decade has noticeably witnessed a remarkable surge in reception and off course public interest in the natural therapies both in the developed and developing countries, it has been estimated that up to 4 billion people (which represents 80% of the population of the world.) living in the developing part of the world are known to rely on herbal medicine products as their primary source of healthcare and traditional medicine practice which entailss the use of herbs is viewed as an integral part of the culture in those communities (Mukherjee, 2002; Bodeker et al, 2005; Bandaranayke, 2006.

Garcinia, a genus belongs to the family Clusiaceae, it consists of very important fruits and medicinal tree species. Majority of these remains in the wild semi-domesticated typess of regional importance however, have been re-discovered as so-called under-utilized crop. *Garcinia kola* heckles generally referred to as bitter kola plays a vital role in African ethno medicine and even traditional ceremonies. The most valued product is the seeds, commonly chewed by both rural and urban population to avoid and treat gastric problems or simply for their astringent taste. The kernel contains a wide range of useful phytochemicals, e.g. high content of tannis and flavonoids. The bioflavonoid kolaviron complex is most discussed. The complex reputedly holds neuro-protective, anti-inflammatory, anti-malaria and wound healing properties. *Garcinia kola* (Bitter kola) is a dicotyledonous plant belonging to the family of Clusiaceae or Guttiferae family. It is a perennial crop growing in the forest, distributed throughout West and Central Africa. *G. kola* is also found in the forest zone of Sierra Leone, Ghana, Cameroon and other West African countries. In Nigeria, it is common in the South Western states and Edo State. Its natural habitat is subtropical or tropical moist lowland forests (Oforkansi et al; 2008).

It is estimated that 20-30% of humans are carriers of *S.aureus* which are known to be part of the normal skin flora, in the nostrils and as a normal inhabitant of the lower reproductive tract of women. *S.aureus* can cause a wide range of illnesses; from minor skin infections, such as impetigo pimples, cellulites, boils, carbuncles, folliculitis, abscesses, scalded skin syndrome and life threatening diseases conditions such as meningitis pneumonia, endocarditis menigitis, osteomyelitis, bacteremia toxic shock syndrome and as well as sepsis (Ugwu etal 2016).

Amongst the ways this can happen include; eating ground meat that was improperly cooked enough to kill the bacteria, untreated milk, vegetables and fruits that have been tainted by water, swallowing water that contains *E.coli,* from animals. (Anie 2019).

The purpose of this study is to assess the antibacterial interact of action of crude methanol extract of *Garcinia kola* seed with Azithromycin. Coetaneous use of orthodox and herbal medicine is practiced by several people in many rural and urban areas in Africa which include many communities and cities in Nigeria. There is a tendency that certain interactions may have taken place without detection in persons who have the habit of consequently using orthodox medicine and herbal drugs.

MATERIALS AND METHOD

Materials

The materials used include; Sterile bottles (Bijou), Test tubes, Sterile Syringes (2ml and 5ml), Sterile swab sticks, Sterile petri dishes, Test tube rack, spatula, wire loop, spirit lamp, cork borer (6mm), muslin cloth, cotton wool, white transparent bucket, conical flask, aluminum foil, measuring cylinder (100ml), masking tape, sterile water, disposable gloves, face masks, autoclave, *Garcinia kola*, incubator (Leader CE MODEL: GP/50/CLAD/250/HID), weighing balance, microscope, refrigerator, hot air oven.

Antimicrobial Agent

Azithromycin (Globela pharma Pvt, India)

Reagents

The reagents used include methanol and disinfectant.

Media

Mueller-Hinton agar (HiMedia laboratories), nutrient broth (HiMedia laboratories), nutrient agar (HiMedia laboratories), mannitol salt agar, and MacConkey agar.

Microbial Culture

Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Streptococcal pyogenes.

Collection of Sample

The seed of *Garcinia kola* (bitter kola) were purchased from Abraka main market, Abraka, Ethiope East Local Government Area of Delta State and authenticated in the Department of Pharmacognosy and Traditional Medicine, Delta State University, Abraka.

Preparation of Methanoic Extract of *Garcinia kola*

The bark of the seeds of *Garcinia kola* was peeled and cut into pieces, air dried and stirred frequently to achieve uniform drying for 14 consecutive days and then pulverized using a mechanical blender. 135g of the fine powder was extracted with 500ml of 80% methanol and 125ml distilled water, each using cold maceration for 5 days and also swirled morning and evening for those 5 days. The extract was filtered using a muslin cloth and allowed to evaporate to a semi-solid residue using a hot air oven. The semi-solid residue obtained were weighed, 6.3g which gave a percentage yield of 4.60% and the extract were stored at 4^0c in a refrigerator (Anie etal 2011).

Preparation of Plant Extract

2g of the extract was dissolved in 40ml of suitable solvent (methanol) to produce a 5% weight per volume extract solution. It was shaken thoroughly prior to preliminary phytochemical screening.

Phytochemical Screening of Bitter Kola *(Garcinia kola)*

The ground bitter kola was subjected to phyto chemical tests for plant secondary metabolites such as saponins, alkaloids, tannins, flavonoids, phenols, terpenoid and steroids and cardiac glycosides.

Preparation of Innoculum

An overnight broth culture was prepared by weighing 0.3g of nutrient broth, using a weighing balance and dissolved in 20ml of distilled water; the media solution was sterilized at 121^0c for 15 minutes in an autoclave and poured into a four sterilized Bijou bottles.

After sterilization, the four Bijou bottles were allowed to cool after which a flamed wire loop was used to inoculate the organisms namely (*Staphylococcus pyogenes, Pseudomonas aeruginosa)* into the Bijou bottles were incubated at a temperature of 37^0c for 24 hours (Anie 2020).

Serial Dilution of Extracts of *Garcinia kola*

For the serial dilution of *Garcinia kola* extract, six test tubes were sterilized previously and 1.2g of the methanol extract was weighed into a test tube containing 4ml of sterile water and was shaken vigorously until the extract was completely dissolved. The remaining 5 test tubes, 2ml of sterile water were measured into them using a sterile syringe. 2ml of the dissolved extract was then transferred from the first test tube into another test tube containing 2ml of sterile water, this was shaken and 2ml transferred also from it into the next test tube. This process was repeated until the last test tube where 2ml was discarded from such that each test tube had 2ml of the solution in it. The concentrations of the extract in each of the test tubes were 300, 150, 75, 37.5, 18.75 and 9.375 mg/mL respectively.

Determination of Zone of Inhibition

The sensitivity of the Isolates [*Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes]* to methanol and aqueous extract of *Garcinia kola* and Azithromycin was determined using agar well diffusion method; eight sterile petri dishes

labeled according to the different concentration were placed and 5oml of sterilized Mueller-Hinton agar was allowed to cool and was poured into each of the petri dishes and allowed to solidify. They were labeled correctly, with each organism having a duplicate. With the aid of sterile swab stick, they were able to collect the organism and swabbed correctly on the respective petri dishes containing the organisms (*Staphylococcus pyogenes, Pseudomonas aeruginosa).* A 6mm sterile cork borer was used to bore holes in the solidified agar on each petri dish. Using a 2ml sterile syringe, 0.5ml of each concentration of the extract was added to their respective holes in the petri dish. The test was repeated for all the organisms, and two replicate tests were performed. The control used against each test organisms was methanol (negative control) and Azithromycin (positive control). After 30 minutes, all the petri dishes were carefully packed with the aid of a masking tape and incubated for 24 hours at a temperature of 37^0c. After 24 hours of incubation, the zone of inhibition around each hole was observed, measured and recorded (Anie etal 2015).

Determination of Minimum Inhibitory Concentration of Extract of *Garcinia kola*

The MIC (Minimum Inhibitory Concentration) of *Garcinia kola* was carried out to ascertain the lowest concentration of *Garcinia kola* extract that can inhibit the visible growth of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus pyogenes.* This process was done by using agar dilution method. A sterile dilution of *Garcinia kola* extract was made with sterile water, 1.2g of the extract to obtain the various concentrations such as 300, 150, 75, 37.5, 18.75, 9.37, 4.68 and 2.34mg/mL. A Mueller-Hinton agar was prepared and transferred to each of the labeled sterile petri dishes. A volume of each of the concentration, which is also equal to 1ml, was also transferred into the labeled sterile petri dishes and made up to 20ml of Mueller Hinton agar. It was then swirled and allowed to solidify. After solidification, each of the plates labeled with different concentrations were divided into 4 segments and also labeled according to each organism. The surface of the agar was then streaked with the isolates using a wire loop. The petri dishes were properly covered and packed and incubated for 24 hours at a temperature of 37^0c. After 24 hours incubation, the least concentration of each of the plant extract that inhibited the growth of the test organism was taken as the MIC (Clinical and labouratory standards institute, CSLI 2012).

Determination of Minimum Inhibitory Concentration (MIC) of Azithromycin

The MIC of Azithromycin was carried out to find out the lowest concentration of the Azithromycin that can inhibit the visible growth of *Escherichia coli, Staphylococcus aeruginosa* and *Streptococcus pyogenes.* Serial dilutions of Azithromycin were carried out, about 8 test tubes were used, and were properly labeled, according to their respective concentrations which are 300, 150, 75, 37.5, 18.75, 9.37, 4.68 and 2.34 (mg/mL). 1ml of Azithromycin was measured into the first test tube and also 1ml of sterile water was measured into it with the aid of a sterile syringe. It was shaken and 1ml was transferred into the next test tube. This process was repeated for the remaining test tubes and 1ml was discarded do that each of the test tubes will contain 1ml. An overnight broth of the test organism (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus pyogenes*) was prepared. 0.1ml of the broth of *Staphylococcus aureus* that was prepared was measured into each test tube containing the different concentration of Azithromycin with the aid of a sterile syringe. The procedure was repeated for the other test organisms. The test tubes were covered with foil paper and left to stand for 24 hours. The MIC was taken to be the lowest concentration which showed no visible growth (Anie etal 2020).

The Evaluation of Interaction Between *Garcinia kola* Extract and Azithromycin Against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus pyogenes*

To ascertain the interaction between *Garcinia kola* and Azithromycin, Mueller Hinton agar was prepared and autoclaved at a temperature of 121^0c for fifteen (15) minutes. Before pouring 20ml into the petri dish and allowing it to solidify, the media was allowed to cool. The serial dilutions of *Garcinia kola* extract and Azithromycin were made to get the MIC's and also to evaluate the combined effectiveness against the various organisms. About 8 sterile petri dishes were used. The procedure was done in duplicates and labeled as Azithromycin, *Garcinia kola* extract in duplicate at the top and bottom of petri dishes, and the organism accordingly labeled at the middle of the petri dish. The test organisms were then smeared with the aid of sterile swab sticks on their respective plates, after which a 6mm cork borer, four (4) wells were punched into each of the plates. The extract of *Garcinia kola* and the Azithromycin with their exact concentration obtained as their MIC well placed in wells, properly labeled and the plates were incubated for 24 hours at 37^0c. After incubation, the zones of inhibition was noted with the aid of a metric rule placed across it and measured from

one edge of the zone to the other edge and the results of the diameter were recorded in millimeters. The combined effects of the antimicrobials on the test organisms were gotten and also recorded Okafo etal (2019).

Determination of the Combined Effect of Methanol Extract of *Garcinia kola* and Azithromycin against *Pseudomonas aeruginosa,* Using the Checkerboard Method

This method is used to determine the impact on potency of the combination of antibiotics in comparison of the individual activities. This comparison is then represented as the Fractional Inhibitory Concentration (FIC) index value. The concentration used ranges from $\frac{1}{256}$ X MIC to 4 X MIC, using a two-fold serial dilution for the checkerboard method. The two agents used for combination were bitter kola extract and Azithromycin in varying ratios ranging from 0:10 to 10:0 of bitter kola extract and Azithromycin. The comparison is represented as:

Fractional Inhibitory Concentration Index (FIC_i) is defined by the equation (Lewis et al, 2002;

Vincent, 2005) as;

$$FIC_i = FIC_A + FIC_B = \frac{MIC_A\ Combined}{MIC_A\ alone} + \frac{MIC_B\ Combined}{MIC_B\ alone}$$

Where;

MIC_A = MIC of Azithromycin

MIC_B= MIC of bitter kola extract.

Note;

If FIC_i < 1 = Synergism.

If FIC_i = 1 = Addictive.

If FIC_i > 1 but < 2 = Indifference, and

If $FIC_i \geq 2$ = Antagonism.

CALCULATION OF PERCENTAGE YIELD OF *Garcinia kola* SEED EXTRACT

$$\text{Percentage yield} = \frac{Final\ weight}{Initial\ weight} \text{ X } \frac{100}{1}$$

For methanol extract

Weight of powdered plant material = 135g

Weight of methanol extract = 6.3g

$$\% \text{ yield} = \frac{6.3g}{135g} \text{ X } \frac{100}{1}\%$$

= 4.66%

RESULTS

Table 1: Bio-chemical test of *S.aureus, E. coli, P.aeruginosa* and *S.pyogenes.*

Biochemical Test	*S.aureus*	*E.coli*	*P.aeruginosa*	*S. pyogenes*
Gram staining	Shape: cocci Color: purple (Positive)	Shape: rods Color: red (Negative)	Shape: Rod Color: blue (Negative)	Shape: cocci Color: white-grayish (Positive).
MacConkey test	Negative	Positive	Positive	Negative
Catalase	Positive	Positive	Positive	Positive
Indole	Negative	Positive	Negative	Negative
Mannitol test	Positive	Positive	Negative	Positive
Motility test	Negative	Positive	Positive	Negative

From table 3. shows the results of biochemical test of *S.aureus*, *E.coli*, *P.aeruginosa* and *S.pyogenes* which gave the following colors and shapes under the microscope- cocci and purple, rods and red, rods and blue, cocci and white-grayish. *S.aureus* and *S.pyogenes* are gram positive organisms while *E.coli* and *P.aeruginosa* are gram negative organisms. The observations of other biochemical tests such as catalase, motility and indole are also shown above.

Table 2: Qualitative analysis results revealing phytochemicals present in the methanol of *Garcinia kola* seed extract.

S/N	Phytochemical constituents	Methanol extract
1.	Saponin	+
2.	Alkaloid	+
3.	Tannins	+
4.	Flavonoids	+
5.	Terpenoids	+
6.	Steroids	+
7.	Cardiac glycosides	-

KEY

+ Presence of secondary metabolite

- Absence of secondary metabolite

From the result obtained in the phytochemical screening of *Garcinia kola* extracts above, it shows that Saponin, Alkaloid, tanins, flavonoids, terpenoids and steroids were present in the methanol extract of *Garcinia kola* while flavonoids and cardiac glycosides were absent.

Table 3: Zone of Inhibition of methanol extracts of *Garcinia kola* seed.

Concentration (mg/ml)	Zone of Inhibition (mm)			
	S.aureus	***E.coli***	***P.aeruginosa***	***S.pyogens***
300	13.5	-	15	5
150	12	-	12	4.5
75	9.5	-	9	3.5
37.5	9.5	-	10	-
18.75	8	-	9.5	-
9.375	8	-	7	-
Azithromycin (+ control)	-	21	33	14.5
Methanol (- control)	-	-	-	-

Reveals the clear zone of inhibition of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes,* which was measured in diameter and indicates the susceptibility of the above test organism to *Garcinia kola* seed extract.

From the table above, it is observed that the largest zone of inhibition for *S.aureus* is (13.5mm) occurring at a concentration of 300mg/ml while the least zone of inhibition (8mm) which occurred in (18.75 and 9.375mg/ml). For *E.coli,* there was no zone of inhibition observed. For *P.aeruginosa,* the largest zone of inhibition (15mm) was observed at 300mg/m, while the least zone of inhibition (7mm) occurred at a concentration of 9.375mg/ml. For *S.pyogenes,* the largest zone of inhibition (5mm) was observed at 300mg/ml, while the least zone of inhibition (3.5mm) occurred at a concentration of 7.5mg/ml. The positive control used was Azithromycin which gave varying zones of inhibition for each for each of the micro-organisms used. *S.aureus* gave no zone of inhibition, *E. coli* was 21mm, *and P.aeruginosa* was 33mm while *S.pyogenes* was 14.5mm. For the negative control which was methanol, there was no zone of inhibition.

Table: Shows the Minimum Inhibitory Concentration (MIC) of methanol extract of *Garcinia kola* against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus pyogenes.* The MIC of the methanoic extract of *Garcinia kola* against *S.aureus* is 2.3437mg/ml, *E.coli* is 300mg/ml, *P.aeruginosa* is 2.3437mg/ml and *S.pyogenes* is 150mg/ml.

Table 4: Minimum Inhibitory Concentrations of methanol extracts of *Garcinia kola* against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus pyogenes.*

Concentration (mg/ml)	Minimum Inhibitory Concentration			
	S.aureus	*E.coli*	*P.aeruginosa*	*S.pyogenes*
300	-	-	-	-
150	-	+	-	-
75	-	+	-	+
37.5	-	+	-	+
18.75	-	+	-	+
9.375	-	+	-	+
4.687	-	+	-	+
2.3437	-	+	-	+
1.1768	+	+	+	+

Key:

+ Growth

-No growth

Table 5: Minimum Inhibitory Concentration (MIC) of Azithromycin against; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus pyogenes.*

Concentration (mg/ml)	Minimum Inhibitory Concentration			
	S.aureus	*E.coli*	*P.aeruginosa*	*S.pyogens*
40	-	+	-	-
20	-	+	-	-
10	-	+	-	-
5	-	+	+	-
2.5	-	+	+	-
1.25	+	+	+	+

Key:

+ Growth

-No growth

Table 3.4b above gives the Minimum Inhibitory Concentration of Azithromycin against *S.aureus* 2.5mg/ml, *E.coli* no MIC, *P.aeruginosa* 10mg/ml and *S.pyogens* 2.5mg/ml.

Table 6: The evaluation of interaction between *Garcinia kola* seed extract and Azithromycin against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus pyogenes.*

Organisms	Concentrations (mg/ml)		Zone of Inhibition (mm)		
	Extract	Azithromycin	Extract	Azithromycin	Extract+ Azithromycin
S.aureus	2.3437	2.5	13.5	-	19
E.coli	300	-	-	21	29
P.aeruginosa	2.3437	10	15	33	27
S.pyogens	150	2.5	5	14.5	16

In table 6 above, the combined antibacterial effect of the methanol extracts of *Garcinia kola* and Azithromycin is presented. Greater zone of inhibition (19mm) against *Staphylococcus aureus* was observed when the extracts and Azithromycin were used simultaneously compared to their individual sensitivity to *Staphylococcus aureus* and it also occurred in *Escherichia coli* having greater zone of inhibition when combined. But this was different with *Pseudomonas aeruginosa* and *Streptococcus pyogenes* because less zone of inhibition was observed when the extract and Azithromycin were used simultaneously, compared to their individual sensitivity to these test organisms.

Table 7: The evaluation of the combined effect of *Garcinia kola* seed extract and Azithromycin against *Pseudomonas aeruginosa*, using the checker board method.

Drug Combination Ratio (AZI: GKS)	MIC of AZI (mg/ml)	MIC of GKS	FIC of AZI	FIC of GKS	FIC Index	Activity Index	Effect
10:0	40	-	-	-	-	-	-
9:1	1.25	0.1465	0.03125	0.0156	0.04685		SYN
8:2	2.50	0.2920	0.0625	0.0313	0.0938		SYN
7:3	5.00	0.5859	0.1250	0.0625	0.1875		SYN
6:4	10.00	1.1719	0.2500	0.1250	0.375		SYN
5:5	20.00	2.3437	0.5000	0.2500	0.750		SYN
4:6	40.00	4.6874	1.0000	0.5000	1.50		INDIFF
3:7	80.00	9.3748	2.000	1.0000	3.00		ANTAG
2:8	160.00	18.7496	4.000	2.000	6.00		ANTAG
1:9	320.00	37.4992	8.000	4.000	12.00		ANTAG
0:10		9.3748	-	-	-	-	-

MIC of Drug alone= 40 ---------- control

MIC of BITTER KOLA alone= 9.3748 ---------- control

Key:

If Fic_i < 1 = Synergism

If Fic_i = 1 = Addictive

If Fic_i > 1 but < 2 = Indifference

If Fic_i ≥2 = Antagonism

AZI= Azithromycin (Drug)

GKS= *Garcinia kola* seed extract

The table 7 above shows the combined activity of methanol extract of *Garcinia kola* seed extract and Azithromycin against *Pseudomonas aeruginosa.* Synergistic effects were recorded at AZI/GKS ratios of 9:1, 8:2, 7:3, 6:4 and 5:5. An indifferent effect was shown at AZI/GKS ratios of 4:6, while antagonism was exhibited at AZI/GKS combinations of 3:7, 2:8 and 1:9Fig 2: shows the result of the Minimum Inhibitory Concentration (MIC) of Azithromycin against *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes* and *Escherichia coli.*

DISCUSSION

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases as well as ameliorating some of the untoward effects that are often associated with synthetic antimicrobials (Okafo etal., 2019). Antibacterial activity of *Garcinia kola* (Bitter Kola) and *Azithromycin was* against *Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae* was studied Table2, the phytochemical analysis of the extract of *Garcinia kola* seed extract sowed the presence of flavonoids, tannins, steroids, terpenoids, saponin and alkaloid. These phytochemical compounds play crucial roles in the bioactivity of the medicinal plant. (Anie 2020) confirms that flavonoids which are part of the phytochemical constituents of *Garcinia kola* shows a wide range of biological activities, one of which is their ability to scavenge for hydroxyl radicals and superoxide anion radicals and thus promotes good health. This plant has been referred to as a "wonder plant" because every part of it hass been found to be of medicinal importance. The flavonoids also exhibit anti-allergic, analgesic, antioxidant, anti-inflammatory and antiangionic properties. Alkaloids are the largest groups of secondary metabolites in plants that have powerful effects on human and could be used as pain killers. Saponins and alkaloids are more abundant in the extract of methanol than the ethanol (Ukaoma A.A. et al., 2013).

The antibacterial properties of the methanol extract of *Garcinia kola* as shown in Table 3 shows that the extract of *Garcinia kola* seed is very effective against the test organisms used and it is observed that the largest zone of inhibition for *S.aureus* is 13.5mm occurring at a concentration of 300mg/ml while the least zone of inhibition (8mm) which occurred at a concentration of 18.75 and 9.375mg/ml. For *E.coli*, there was no zone of inhibition observed.

For *P.aeruginosa*, the largest zone of inhibition (15mm) was observed at 300mg/ml while the least zone of inhibition (7mm) occurred at a concentration of 9.375mg/ml. For *S.pyogens*, the largest zone of inhibition (5mm) was observed at 300mg/ml while the least zone of inhibition (3.5mm) occurred at a concentration of 75mg/ml. The positive control used was Azithromycin which gave varying zones of inhibition for each of the micro organisms used. *S.aureus* gave no zone of inhibition, *E.coli was 21mm, and P.aeruginosa* was 33mm while *S.pyogens* was 14.5mm. The sensitivity test carried out is used to determine the effectiveness of antimicrobial agents from plant extract and Azithromycin used against microorganisms and the diseases caused by it (Oghenejobo et. al., 2017).

The Minimum Inhibitory Concentration (MIC) result of *Garcinia kola* in table 4 and 5 indicates the *S.aureus* had MIC of 2.3437mg/ml, *E.coli* had an MIC of 300mg/ml, *P.aeruginosa* had an MIC of 2.3437mg/ml and *S.pyogens* had an MIC of 150mg/ml. From the result, it can be deduced that *Garcinia kola* seed extract is effective in inhibiting both gram positive and gram negative organism used above. In table 3.4b, the MIC (Minimum Inhibitory Concentration) of Azithromycin shows that *Staphylococcus aureus* had an MIC of 2.5mg/ml, *Escherichia coli* had no MIC, and *Pseudomonas aeruginosa* had MIC of 10mg/ml while *Streptococcus pyogen* had an MIC of 2.5mg/ml. Azithromycin is a broad spectrum antibiotic, it can be seen from the MIC result gotten, that Azithromycin is highly effective against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* except *Escherichia coli* which has no MIC which indicates from the result that Azithromycin has no effect against *Escherichia coli.*

The combined antibacterial effect of the methanol extracts of *Garcinia kola* and Azithromycin is presented in Table 6. for *Staphylococcus aureus,* greater zone of inhibition (19mm) was observed when extracts and Azithromycin are used simultaneously compared to their individual sensitivity to *Staphylococcus aureus* same as *Escherichia coli* having greater zone of inhibition when combined. But it differs with *Pseudomonas aeruginosa* and *Streptococcus pyogenes* because less zone of inhibition was observed when the extract and

Azithromycin were used simultaneously compared to their individual sensitivity to test organisms. Table 7 shows the combined activity of methanol extract of *Garcinia kola* seed extract and Azithromycin against *Pseudomonas aeruginosa.* Synergistic effects were recorded at AZI/GKS ratios of 9:1, 8:2, 7:3, 6:4 and 15:5. An indifferent effect was shown at AZI/GKS ratio of 4:6, while antagonism was exhibited at AZI/GKS combinations of 3:7, 2:8 and 1:9. The FIC (Fractional Inhibitory Concentration) is interpreted as synergism if its value is less than 1, addictive if it is equal to 1, indifference if it is more than 1, but less than 2 and antagonism if it is more than 2 (Ofokansi et. al., 2008).

The synergistic effect was seen to decrease at the combination of Azithromycin and *Garcinia kola* seed extract when the ratio of Azithromycin was reducing and that of *Garcinia kola* seed extract, it was increasing in the combination. A rational explanation for this enhanced activity in combination is that the antimicrobial principles in methanol extract of *Garcinia kola* seed extract and Azithromycin may possibly have varying mechanisms of action or may be inhibiting two different steps in the same biosynthetic pathway of the organism resulting in an overall synergy at certain combinations (9:1, 8:2, 7:3, 6:4). Azithromycin acts by reversibly binding to the bacterial ribosome and inhibit protein synthesis and a long half-life due to extensive uptake in tissue, particularly lung, tonsil and prostate while the mechanism of action of *Garcinia kola* seed extract is not yet clarified. An indifferent effect was recorded at ratio 4:6, meaning that the combination ratios of *Garcinia kola* seed and Azithromycin have no effect on the stated micro organisms. Antagonism was recorded at ratio 3:7, 2:8 and 1:9.

CONCLUSION

This study provides an indispensable evidence of some kind of antibacterial interaction which is found between the methanol extract of *Garcinia kola* seed and Azithromycin against *Staphylococcus aureus* and *Escherichia coli*. The combinations of Azithromycin and *Garcinia kola* seed extract may pose some importance in the chemotherapy of infections in which *S.aureus*, *E.coli* and also *Pseudomonas aeruginosa* as shown in the Checker Board method, especially when the concentration of the extract of *Garcinia kola* seed is kept relatively low and Azithromycin is high.

REFERENCES

1. Clement O. Anie, Matthew I. Arhewoh and Henry A. Okeri**.** Antimicrobial Activity Of Crude Extracts Of *Diospyros monbuttensis*(Fam: Ebenaceae) Root And Stem Barks, **2011.**
2. P.J. Houghton, "Synergy and polyvalence: paradigms to explain the activity of herbal products". Evaluation of herbal medicinal products, 2009; 85-94.
3. Bin Osman, M; Milan, R. Mangoslen- *Garcinia mangostana;* South Hampton Centre for under-utilized crops, University of South Hampton: Southampton, UK, 2006; ISBN 0-85432-817-3.
4. Christiana Orevaoghene Akpo, Clement Oliseloke Anie and Vivian Orhire *In-Vivo* Antimalarial Activity of the Aqueous Leaf Extract of *Phyllanthus amarus* Schum & Thonn. against *Plasmodium berghei* Infected Mice East African Scholars Journal of Medical Sciences *Abbreviated Key Title: East African Scholars J Med Sci*.
5. Bodeker C., Bodeker G., Ong C.K., Grundy C.K., Burford G., Shein K. WHO Global Atlas of Traditional, complementary and alternative medicine. Geneva, Switzerland: World Health Organization, 2005.
6. Bandaranayake W.M. (2006). "Quality control, screening, toxicity and regulation of herbal drugs" in modern phytomedicine. Turning medicinal plants into drugs Eds Ahmad I., Aqil F., Owais M., editor. (Weinheim: Wiley-VCH GmbH & Co. KGaA;) 25-37 10.1002/97 83527609987.Ch.2.
7. Ugwu MC Anie CO Ibezim EC and Esimone CO. Antimicrobial.Evaluationof Methicillin-Resistant Staphylococcus Aureus Nasal Carriage amongst Healthy Students in Agbor, Delta State, Nigeria. Archives of clinical microbiology, 2016; 7(2): 13.
8. Anie Clement Oliseloke. Antmicrobial susceptibility pattern of Staphylococcus aureus isolated fro cows. Sokoto Journal of Medical Laboratory Science, 2019; 4(2): 35–41.
9. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard, ninth edition, 32: 2.
10. Anie CO, Nwabuokei IG, Oghenejobo M, Enwa FO**.** The Antibacterial Effect of the Leaf Extract of *Buchholzia coriacae(Capparidaceae)* on Gram-Negative Nasal Isolates Scholars Academic Journal of Pharmacy Sch. Acad. J. Pharm., 2015; 4(4): 226-231.
11. Monica Chesebrough. District Laboratory Practice in tropical countries, part two, 2000; 38; 64-72; 137-140; 391-398.

12. Michael Oghenejobo, Opajo A. OOghenejobo Bethel, Uzoegbu U. Antibacterial Evaluation, Phytochemical Screening and Ascorbic Acid Assay of Turmeric (Curcuma longa)MOJ Bioequivalence, 2017; 4: 2.
13. Ukaoma, A.A., Ukaoma, V.C., Okechukwu, B. I.Iwuagu, M.: Journal of phytopharacology., 2013; 2(3): 34-38.
14. K C. Ofokansi, AN Mbanefo, MN Ofokansi, LO Esimone:, Antibacterial interaction of crude methanol extract of G.K. seed with gatifloxacin. Tropical Journal of Pharmaceutical Research, 2008; 7(4). DOI: 10.4314/tjpr.v7i4.14702
15. S. E. Okafo, C. O. Anie, M. C. Nwanua**(2019).** Formulation and Evaluation of Antimicrobial Topical Creams from Ethanol Extract of *Vernonia ambigua* Leaves. Nig. J. Pharm. Res., 2019; 15(2): 249-255.
16. Anie C.O. The Antimicrobial Activities of Root Extract of *Buchholzia coriacea* Engler (Wonderful Kola) against Gram-positive and Gram-negative Pathogens. Sokoto Journal of Medical Laboratory Science, 2020; 5(1): 44–51.
17. Clement Oliseloke Anie, Michael Oghenejobo Esther Imabong Edomokong, Ejemighaye, Tracy. In-vitro antibacterial interaction of crude methanol extract of Zingiber officinale with a quinolone against Escherichia coli, Staphylococcus aureus and Bacillus subtilis International Journal of Biosciences, 2020; 16(2): 31-39.