Antimicrobial Effects of Bitter Kola (Garcinia kola) Nut on Staphylococcus aureus, Escherichia coli and Candida albicans

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Abstract: This study was conducted to test for the antimicrobial effect of Bitter kola nut (Garcinia kola) on Staphylococcus aureus, Escherichia coli and Candida albicans. Garcinia kola seed (nut) were collected from Main market Abakaliki (Abakpa market) and carried to microbiology laboratory complex of Ebonyi State University for laboratory analysis. The Garcinia seed (nut) were dehusked and cut into piece, air dried and grinded into powdered form and 100g each was extracted with 95 % aqueous ethanol, cold and hot water in 500 ml in the ratio of 1:5 (W/V) at room temperature for 24 hours and filtered with Muslin filter cloth. The mixture was filtered in a flat plate and air dried at room temperature. The crude extract was dissolved with Dimethyl Sulphoxide and carried to serial dilution (2 fold serial dilutions). A total of three microbial organism bacteria (staphylococcus and Escherichia coli) and fungi (Candida albicans) obtained from laboratory was screened using biochemical and microbiological standard. The isolates were further tested for susceptibility testing using Muller-Hinton agar by agar well diffusion method. The result of susceptibility test revealed that the garcinia kola extract yielded the highest inhibition zone diameter of 21 mm and 20 mm at 100 mg/ml and 50 mg/ml in ethanol against Staphylococcus aureus while in Candida albicans at 100 mg/ml and 50 mg/ml recorded (20 mm) and (20 mm) in ethanol solvent. The study also revealed that Garcinia kola nut was best extracted with ethanol solvent. It also enhances the bioactive compound or component of the plants. Therefore in this study, Garcinia kola extracts could be used by the pharmaceutical industries to produce drugs that can cure or prevent infectious disease.

Key words: Antimicrobial • Bitter kola • Staphylococcus aureus • Escherichia coli and Candida albicans

INTRODUCTION

Plant derived medicines, which have large contributions to human health and well-being, has provided source of inspiration for novel drugs and served as models for western drugs [1]. It is estimated that over 80% of modern pharmaceutical products are based on herbs. According to World Health Organization (WHO), up to 80% population in Africa depends on traditional herbal medicine for primary healthcare, this accounts for around 20% of the overall drug market. A number of plants that have medicinal and antimicrobial properties in Nigeria have been identified and documented, for instance, artemisinin from Artemisia annua, used in the manufacture of Artisunate and other artemisinin based drugs, which serves as potent anti-malaria drug is a popular drug in the market [2].

Plant derived medicines have many benefits such as; low toxicity status, relative safety, accessibility and affordability, plant part have been a source of herbal medicines which has been shown to be effective to about 80% of population as primary healthcare [3]. One of such plants is Garcinia Kola’ a member of Guttiferae species found throughout West and Central Africa, Every part of Garcinia Kola (bitter kola) is an important component in Traditional herbal medicine World Wide [3].

Garcinia Kola (bitter kola) is a dicotyledonous plant belonging to the family of plant called Guttiferae, It is a perennial crop growing in the forest, distributed throughout West and Central Africa [4]. Bitter kola nut is also found distributed in the forest zones of Sierra Leone, Ghana, Cameroon and other West African countries. In Nigeria, it is common in the South Western States and Edo State [4].

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Therefore, Antimicrobial agent is defined as an agent capable of destroying or inhibiting the growth of microorganism especially pathogenic ones. It is also an agent that kills microorganisms or inhibits their growth. Another definition states that an antimicrobial agent is a substance that fight against or impede the growth and development of microorganisms [5].

Most infections are caused by the individual’s own endogenous flora which is present on the skin, respiratory tracts, intestine (digestive system) and circulatory system. Usual pathogen on the skin and mucous surface are gram-positive cocci namely *Staphylococcus aureus* and Gram-negative aerobic and anaerobic bacteria *E. coli* can cause infections [6]. In other words fungi can also cause infections such as *Candida albicans* on the skin particularly on pregnant women or female private parts.

Therefore it is encouraging to assess the antimicrobial effects of *Garcinia kola* extracts on *S. aureus*, *E. coli* and *Candida albicans*.

**Justification of Study:** The observed increase in antibiotic resistance among the strains of *Staphylococcus aureus* (MRSA) *Escherichia coli* and *Candida albicans* in our hospitals and community is alarming hence the need for this research work. We will evaluate the organisms as mentioned above to know their antimicrobial activities against the plant extract.

**Research Question:** Will the antimicrobial activities of the bitter kola nut extract have effect on the test organisms.

**Aim and Objective**

**Aim:** To assess the antimicrobial effects of *Garcinia kola* extracts on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

**Specific Objectives:** To determine the antimicrobial activities of cold water, hot water and ethanol plant extracts on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

To determine the effect of these plants extract against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

To determine the minimum inhibitory concentration (MIC) *Garcinia* extracts against clinical isolate of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

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**MATERIALS AND METHODS**

**Materials:** The materials used was *Garcinia kola* (Bitter kola).

**Equipments/Instruments:** The equipment/instruments used were the following.

Measuring cylinder, beaker, conical flask, petri dishes, spatula, meter rule, Pasteur pipettes, distilled water, wire loop, Biju bottles, swab sticks, markers, cotton wool, masking tapes, wooden stick, aluminum foil and cork borer, microscope, (Olympus Germany) incubator (Mereck Germany), Weighing balance, autoclave, colony counter machine, refrigerator and Bunsen burner and Grinding machine.

**Culture Media:** The media used in this work include nutrient agar (T.M. Media India), MacConkey agar (T.M. Media India), Nutrient broth and Miller Hinton agar (T.M. Media India). They were prepared according to the manufacture’s instruction.

**Chemicals/Reagent:** The following chemicals were used in the work. Crystal violet, lugol’s iodine, acetone/alcohol, safranine, Kovac’s reagent (fischer scientific company, USA) oxidase reagent (tetramethyl-p-then lendediamine) immersion oil, hydrogen peroxide, distilled and normal saline and dimethyl sulphoxide (99%).

**Methods**

**Study Area:** The sample collection of this study was carried out at Abakaliki in Ebonyi State South East Nigeria. The sample was analyzed at Ebonyi State University Microbiology Laboratory complex.

Ebonyi State (Abakaliki) is located between latitude 6°.20N and longitude 8° 06°E and rainfall pattern is biomodal (April-July) and September – November with a short spell at some times in August. The annual rainfall is between 1000 mm-1500 mm. The plantation is predominantly, savannah, the mean annual temperature is about 24°C and the relative humidity is between 60-80% [6].

According to the National population census [7] the population of people in Abakaliki is about 1, 064, 156 males and 1, 112, 791 females. There is an estimated population of 4.3 million people in Ebonyi State the land mass of Ebonyi State is 5, 935km². The occupation of people in the area is mainly farming and trading, though there are also civil servants and students.
Sample Collections: A total of 200 bitter Kola (Garcinia kola) was bought from main market in Abakaliki metropolis. The samples were air dried and brought to the Applied Microbiology Laboratory complex, Ebonyi State University Abakaliki in a sterile polyethylene bags where they were analysed using standard microbiology techniques.

Sample Preparation
Ethanol Extract: The seed of Garcina kola were dehusked and cut into pieces, air dried and grinded with manual grinding machine into powered form (Gladhill, 1977). The air dried and grinded Garcina kola nuts (100g) each was extracted with 95 % aqueous ethanol (500 ml) in the ratio 1:5 (W/V) at room temperature for 24 hours. The mixture was filtered and the filtrate was allowed to evaporate to dryness and was kept in deep freezer until needed (Aishamma and Mitscher, 1979).

Cold Water Extract: A 100g of each of the powdered garcina kola nut was weighted and soaked into 500 ml of cold distilled water in a conical flask and covered with aluminum foil paper and sealed with mask tape. The mixture was shaked and allowed to stand on the bench for 24 hours. After, it was filtered using Musilin cloth. The filtrate was allowed to evaporate to dryness.

Hot Water Extract: A 500 ml of water was boiled using a Bunsen burner after which, the 100g of the weighed Garcina kola nut was soaked into the 500 ml in conical flask and covered with aluminum foil paper. The mixture was shake and allowed to stand for 24 hours. After which it was filtered using musilin cloth and allowed to evaporate to dryness.

Test Organisms: The test organisms used were Escherichia coli, staphylococcus aureus and candida albicans. These organisms were isolated from already culture organism from the laboratory unit of Applied Microbiology Department in Ebonyi State University Abakaliki.

Identification of Isolates: The following test were carried out to confirm the test organism as described by Cheesbrough (2006), they include Gram staining, catalase test, coagulase test and indole test Gram staining.

Gram Staining: A smear of the isolate was prepared by placing a drop of distilled water on a clean slide. The test organism was picked with sterile wire loop and mixed with the distilled water on the slide and allowed to air dry. The smeared slide was fixed by passing it three times over a Bunsen burner flame. The fixed smears were covered with crystal violet stain for 1 minute and was washed a off with tap water. The smears were covered with Lugol’s iodine for 1 minute and washe off with clean tap water. They were decolourized with acetone and rinsed off immediately with water.

Safranin was used to cover for 1 minute and rinsed with tap water and allowed to air dry on drainage rack. Slides were examined microscopically using 100x objective lens [8].

Biochemical Test
Catalase Test: The test organism was collected with sterile wire loop and immersed on a drop of normal saline on a slide. One drop of 3 % hydrogen period was added and observed for the formation of air bubbles [9]. Presence of air bubbles resulting from produce of oxygen gas within 5-10 seconds indicated positive result.

Coagulase Test: Two drops of distilled water were placed on each end of the slide and colony of the test organism was emulsified in each of the drops to make stick suspension. A loopfull of plasma was placed on one of the suspension and mixed thoroughly and observed for clumping, no plasma was added to the second suspension in order to differentiate any granular appearance of the organism from true coagulase. Presence of clumping indicate positive result. Absence of clumping shows negative result. Sensitivity testing is also used for the diagnosis [10].

Indole Test: A 5 ml of nutrient broth was put into test tubes and sterilized, allow to cool. Wire loop was used to collect the colony of test organism and inoculate and incubate for 72 hours. After that, 2-3 drops of Kovack reagent was put into the test tube containing broth, within 10 mins formation of red/pink colour indicates the positive result.

Test for Candida albicans
Cultural Identification: Sabourand Dextrose corn meal agar medium was prepared aseptically. A 1 % of tween 80 was added to corn meal agar and wire loop was used to collect the test organism and inoculate in the media, incubate at 35°C - 37°C for 48 hours. After incubation, the plate was examined for colony growth.
Gram Staining: A smear of the colonies were made on clean grease free slide and allowed to dry. Heat fix was made and stained with Gram staining reagents. It was examined under microscope with 100x objective lens. A single budding of the cell was seen attached to pseudohyphae. Both the budding of the cell and pseudohyphae are gram positive for the *candida albicans*.

Confirmatory Test for *Candida albicans*

Germ Tube Test

**Principle:** Germ tube test is the confirmatory test for *candida albicans*. The test are short outgrowth, non-septate germinating hyphae. They are ½ the width and 3-4 times length of the cell from which they arise. When cells of candida are incubated in serum at 37°C for 2-4 hrs candida albicans produce short, slender tube like structures called germ tubes. Formation of germ tube is associated with increased synthesis of protein and ribonucleic acid.

**Procedures:**

- Aliquot of 0.5ml (12drops) of serum or media in a test tube.
- Make a light suspension of the suspect yeast colonies (by touching 1-2 large colonies or 3-4 smaller) colonies with a sterile wooden applicator on serum.
- Incubate the tube for 2-3 hours in a 35-370C incubator.
- Place a drop of the suspension on a slide using a pasteur pipette and cover with a coverslip.
- Examine the wet mount microscopically (at 40x) for production of germ tubes (long tube like projections extending out from the yeast cells).
- Result and interpretation
  - Positive result shows a short hyphal (filamentous) extension arising laterally from a yeast cell with no constriction at the point of origin. Is confirmed as candida albicans.
  - No hyphal extension arising from a yeast cell or short hyphal extension with constriction at the point of origin indicates Negative result.

**Standardization of the Tests Organism:** The test organism was standardized by the use of 24 hours old broth cultures prepared by inoculating the test organism into 5 ml of nutrient broth and the culture was adjusted to obtain 0.5 McFarland turbidity equivalent standards [12].

Antimicrobial Susceptibility Test: The susceptibility patterns of bacteria *E. coli* and fungi pathogen isolated from the laboratory was determined by Kirby-Bauer susceptibility test method as recommended by the [13]. Clinical Laboratory standard institution. A 24 hours culture of the test organism grown in nutrient broth was adjusted to 0.5 McFarland turbidity standard. The inoculums were aseptically inoculated on the surface of Muller-Hinton agar plates using sterile swab sticks. Commercially available single antibiotic disk namely.

Azithromycin (AZM) and fluoconazole (25µg)/Amphotericin B (Oxoide, Uk) were aseptically impregnated on the surface of inoculate Muller-Hinton agar plates and Muller-Hinton agar supplemented with 2 % glucose and methylene blue. The plate were incubated at 37°C for 24 hrs and the inhibition zone diameter (IZD) produced by the antibiotic disks was measured with a meter rule and recorded as recommended by [14].

Activities of Plant Extracts: Antimicrobial susceptibility plant extracts was determined by the method of [15] using agar well diffusion method. The test organism was subculture into fresh nutrient broth. The broth culture was then incubated at 37°C to achieve the turbidity of 0.5 MacFarland standards.

A 15-20 ml of molten Muller-Hinton agar was asexptically poured into sterile petri dishes of equal sizes and allowed to gel or solidify. The test organism was aseptically inoculated on the surface of solid Muller-Hinton (MH) agar plates using sterile swab stick.

A well of 8 mm in diameter was punctured or bored holes in the cultured media with sterile cork, borer, 0.5 ml of the plant extracts was used to fill the holes. The plates were incubated at 37°C for 24 hours (bacteria) while 72 hours (fungi). The zone of inhibition produced was measured with meter rule as recorded by [16].

**RESULTS**

A total number of three already provided isolates obtained from microbiology laboratory was analysed using standard microbiological methods. The isolates were identified based on Gram staining test and biochemical test which includes catalase test, coagulase test, indole test. The biochemical test carried out both catalase and coagulase test revealed positive on *S. aureus* while indole test reveal positive on *E. coli*. Gram staining revealed purple colour in *S. aureus*, red/pink colour in *E. coli* while in *candida albicans* white to opaque switching and budding of the cell attached to
Table 1: Morphological, microscopic and Biochemical characterization of bacterial and fungi isolate

<table>
<thead>
<tr>
<th>Sample</th>
<th>Colony characterization</th>
<th>Gram Rxt</th>
<th>Biochemical Catalase Test</th>
<th>Coagulase</th>
<th>Suspected Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code/S/N</td>
<td>Purple, cocci, clustered, yellow, creamy and smooth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>S. aureus</td>
</tr>
<tr>
<td>2</td>
<td>Red/Pink, smooth glossy</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>E. coli</td>
</tr>
<tr>
<td>3</td>
<td>White to opaque in colour, creanated and flat, pseudohyphae and buds</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>C. albicans</td>
</tr>
</tbody>
</table>

Key = + positive, – V = Negative, S – staphylococcus aureus E. coli Escherichia coli and C. albican = Candida albicans.

Table 2: The Inhibition Zone Diameter of Plant Extracts of Garcinia kola (Bitter Kola) against Staphylococcus aureus in different Solvent

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Concentration (mg/ml)</th>
<th>Inhibition zone diameter (mm)</th>
<th>Control AZM (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold water</td>
<td>100 mg/ml</td>
<td>13 mm</td>
<td>24 mm</td>
</tr>
<tr>
<td></td>
<td>50 mg/ml</td>
<td>12 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 mg/ml</td>
<td>9 mm</td>
<td></td>
</tr>
<tr>
<td>Hot water</td>
<td>100 mg/l</td>
<td>12 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 mg/ml</td>
<td>12 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 mg/ml</td>
<td>8 mm</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>100 mg/ml</td>
<td>21 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 mg/ml</td>
<td>20 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 mg/ml</td>
<td>17 mm</td>
<td></td>
</tr>
</tbody>
</table>

Key: AZM – Azithromycine (24µg)

Table 3: The Inhibition Zone Diameter of Plant Extracts of Garcinia kola (Bitter kola) against Escherichia coli in different Solvent and Concentrations

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Concentration (mg/ml)</th>
<th>Inhibition zone diameter (mm)</th>
<th>Control AZM (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold water</td>
<td>100 mg/ml</td>
<td>13 mm</td>
<td>29 mm</td>
</tr>
<tr>
<td></td>
<td>50 mg/ml</td>
<td>12 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 mg/ml</td>
<td>8 mm</td>
<td></td>
</tr>
<tr>
<td>Hot water</td>
<td>100 mg/l</td>
<td>12 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 mg/ml</td>
<td>8 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 mg/ml</td>
<td>7 mm</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>100 mg/ml</td>
<td>10 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 mg/ml</td>
<td>9 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 mg/ml</td>
<td>7 mm</td>
<td></td>
</tr>
</tbody>
</table>

Key – AZM = Azithromycin, E. coli – Escherichia coli

psuedohyphae on microscopic examination using 100x objective lens. Gram positive cocci and clustered arrangement while E. coli was rod shape. Candida albicans was in pseudo hyphae braches with buds which were observed and recorded.

Table (2) the inhibition zone diameter of plant extracts of Garcina kola (bitter kola) against S. aureus in different solvent and concentrations. The highest concentration of the G. kola extract was at 100 mg/ml and 50mg/ml in Ethanol (21 mm and 20 mm and in cold water 13 mm and 12 mm) while in Hot water 12 mm and 12 mm but at 25 mg/ml the inhibition zone diameter in ethanol 17 mm, in cold water 9 mm and hot water 8mm.

Table (3) shows the inhibition zone diameter of plant extracts of G. kola against Escherichia coli in different solvents and concentration. The highest inhibition zone diameter of cold water extracts of G. kola gave 13 mm at 100 mg/ml while at 50 mg/ml 12 mm and at 25 mg/ml 8 mm was recorded. In Hot water it yielded 12 mm at 100 mg/ml while at 50 mg/ml 8 mm and at 25 mg/ml was recorded 7 mm. The lowest inhibition zone diameter was observed in ethanol extracts which yielded 10 mm at 100 mg/ml at 50 mg/ml gave 9 mm while at 25 mg/ml it recorded 7 mm.

Table (4) revealed the inhibition zone diameter of plant extract of Garcinia kola against candida albicans. The highest inhibition zone diameter of cold water at 100 mg/ml yielded 13 mm, at 50 mg/ml it gave 12 mm while at 25 mg/ml 12 mm. In Hot water at 100 mg/ml it yielded 12 mm, at 50 mg/ml it gave 12 mm while at 25 mg/ml it recorded 11 mm. In ethanol at 100 mg/ml it gave 20 mm at 50 mg/ml it yielded 20 mm while at 25 mg/ml it recorded 17 mm. It was observed that among the three organisms the highest inhibition zone diameter was recorded in Candida albicans in comparing to the control drugs used.

The result of the minimum inhibitory concentration of the seed garcina kola extracts against test organism showed that the extracts was effective when compared with the zones of inhibition of the standard antibiotics used (Table 5) this agrees with [17] who reported that G. kola seed extracts exhibited bacterial effects very well compared to standard antibiotics.
Table 4: The inhibition zone diameter of plant extracts *Garcinia kola* against *Candida albicans* in different solvent and concentrations

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Concentration (mg/ml)</th>
<th>Inhibition zone diameter (IZD) mm</th>
<th>Control fluoconazole 25 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold water</td>
<td>100 mg/ml</td>
<td>13 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 mg/ml</td>
<td>12 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 mg/ml</td>
<td>12 mm</td>
<td></td>
</tr>
<tr>
<td>Hot water</td>
<td>100 µg/l</td>
<td>12 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 mg/ml</td>
<td>12 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 mg/ml</td>
<td>11 mm</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>100 mg/ml</td>
<td>20 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 mg/ml</td>
<td>20 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 mg/ml</td>
<td>17 mm</td>
<td></td>
</tr>
</tbody>
</table>

Key – AZM = Azithromycin, *E. coli* – *Escherichia coli*

Table 5: Minimum inhibition concentration (MIC)

<table>
<thead>
<tr>
<th>Plant Concentration extracts mg/ml</th>
<th>Solvent</th>
<th>Inhibition zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>100 mg/ml</td>
<td>Cold water</td>
<td>Nil</td>
</tr>
<tr>
<td>50 mg/ml</td>
<td>Hot water</td>
<td>Nil</td>
</tr>
<tr>
<td>25 mg/ml</td>
<td>Ethanol</td>
<td>25</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Antibiotic resistance is a great global concern. The increasing failure of chemotherapeutic and antibiotic resistance exhibited by pathogenic microbial agents has led to the screening of plants for their antimicrobial activity.

A total number of three (3) isolates obtained from microbiology laboratory were identified based on preliminary and biochemical tests. The result were Gram positive cocci and clustered arrangement, purple, colour (*Staphylococcus aureus*), red/pink colour, rod like (*Escherichia coli*), white to opaque switching, flat with budding cell attached to pseudohyphae (*Candida albicans*). The result is in line with [18, 19] who reported that *S. aureus* is the normal flora of skin urogenital tracts, gasterointestinal and colonic flora which causes both surgical, accidental, respiratory gasterointestinal and colonic infections.

In this study, the result obtained showed that the plant used in the study have bioactive compound against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. This is in agreement with [20] who reported that medicinal plants possesses antimicrobial activity. Antimicrobial activities of the medicinal plant used was determined using agar well diffusion method as described by [21].

Azithromycine and fluoconazole was used as positive while distilled water was used as negative control. In table 2: the inhibition zone diameter of plant extracts showed that at 100 mg/ml, the plant extract of *Garcina kola* was active. The plant extracts inhibited the growth of the isolates (*S. aureus*) in cold water (13 mm), Hot water (12 mm), Ethanol (21 mm) and at 50 mg/ml (12 mm) in cold water, in Hot water (12 mm) and in ethanol (20 mm) while at 25 mg/ml cold water (9 mm) Hot water (8 mm) and in ethanol (17 mm). The result obtained indicates that different solvent in extracts have different compound with antimicrobial activity. This suggest that the antibacterial activity could be due to different classes of compound or solvent used [22]. Ethanolic extracts had the highest inhibition zone diameter (21 mm) and (20 mm).

This could mean that active ingredient of the plant extracts are not equally soluble to cold water and Hot-water. This is in agreement with the finding of [23] who reported ethanol as the best solvent for the extraction of plant active substance of medical importance.

Table 3 showed the inhibition zone diameter of plant extracts on *Escherichia coli*. It was observed that the lowest inhibition zone diameter was on ethanol 1 extracts which yielded (10 mm) at 100 mg/ml and at 50 mg/ml (9 mm) while at 25 mg/ml (7 mm). This is in agreement with the finding of [24] who reported that *E. coli* survives in moist environment that is faecal pollution (always of water source, drinking water and food contaminant). It also indicates that ethanol help to inhibit its growth due to acidic nature of it. *E. coli* does not thrive well on ethanol solvent because of gram – negative facultative anaerobes [25].

*E. coli* also may be due to the fact that the bacterium possesses mechanism for dexotoxifying or removing the active principles. Table 4 showed the inhibition zone diameter of plant extracts *Garcina kola* against *Candida*
albicans. The highest inhibition zone diameter was observed in ethanol, at 100 mg/ml which gave (20 mm), at 50 mg/ml, it yielded (20 mm) while at 25 mg/ml, it recorded (17 mm). It was observed that the oil in the seed of Garcina kola was dissolved thoroughly by the Dimethyl sulfoxide (DMSO) chemical.

Again, the ethanol solvent was effective on Garcina kola thereby promoting the biological activities of the extracts. According to [26] and [27] who reported that Garcina kola revealed the presence of flavonoids, tannins, cardiac glycoside, steroids, saponins and trace reducing sugar.

According to [28] the reported in his work that flavonoids which are part of the phytochemical constituents of G. kola exhibit a wide range of biological activities, one of which is their ability to scavenge for hydroxyl radicals and superoxide anion radicals and this promotes good health. Flavonoids also exhibits anti-inflammatory, antiangionic and antioxidiant properties. This is in agreement with [29] who reported in his work that antifungal activity of G. kola to the extracts on Candida albicana, aspergillus niger shows susceptibility to the extracts. It was observed that G. kola extracts is the most effective on both bacterial and fungi organisms in comparing to the inhibition zone diameter of the drugs used (Azithromycin (24µg) and fluconazol (25µg). It also agrees with [30] who reported that at higher concentrations, most antifungal exhibit higher antifungal activity. The minimum inhibitory concentration (MIC) of the plant extract against the tested bacteria and fungi isolates was also determined.

The minimum inhibitory concentration of the seed Garcina kola extracts against test organism show that the extracts was effective when compared with the zone of inhibition of the standard antibiotics used (Table 5). This agrees with [31] who reported that G. kola seed extracts exhibited bacterial effects very well compared to standard antibiotics.

**Summary:** In this research work, the importance of utilizing Garcina kola (Bitter kola) as a pharmaceutical option was observed as effective against bacterial and fungi organism (Staphylococcus aureus, Escherichia coli and Candida albicans) isolated from laboratory. The plants extracts showed the inhibition zone diameter of 21 mm at 100mg/ml in ethanol solvent, 13 mm in cold water against Staphylococcus aureus and Escherichia coli comparing to Azithromycin (15µg) which was used as positive control while Candida albicans 20 mm at 100 mg/ml in ethanol and 13 mm in cold water as compared to fluconazol (25µg) as positive control. The seed of the plant was chosen because of its availability and economic medicinal importance.

**CONCLUSION**

The study showed that Garcina kola seed extracts had a concentration which depend on antimicrobial activity against both bacterial and fungi organisms. From the findings in this study, it showed that G. kola is very important in health benefits, food products formulation and pharmaceutical therapeutic values.

**Recommendation:** Considering the finding from this research work it is necessary to recommend that we should make use of medicinal plant around us to treat pathogenic disease and also to reduce multiple drug resistance in our environment today.

Further more, it will be necessary to explain the mechanism of action and as well as their levels of toxicity to assess their clinical application.

Thirdly, from the study, we conclude that medicinal plant like Garcina kola (bitter kola) seed should be used as pharmaceutical by product for production of drugs such as cough, skin infection, stomach ache, gasterotitis, disorder of liver, hepatitis and antiseptic including gram positive and negative organism.

**REFERENCES**