EFFECT OF PLANT EXTRACTS ON Staphylococcus aureus FROM Clarias gariepinus (BURCHELL, 1822) JUVENILES IN LAGOS, OGUN AND OYO STATES, NIGERIA

Department of Aquaculture and Fisheries Management
University of Agriculture, Abeokuta, Nigeria.
adeoluakinyemi@yahoo.com

ABSTRACT
This study aimed at assessing the antibacterial activity of Garlic (Allium sativum), onion (Allium cepa) and bitter kola (Garcinia kola) against Staphylococcus aureus isolated from Clarias gariepinus juveniles from 15 different farms in Oyo, Ogun and Lagos states, Nigeria. Staphylococcus aureus was isolated from the gills, skin and buccal cavity of the fish. The result showed that University of Agriculture, Abeokuta Fish Farm (UNAAB) in Ogun state, recorded the highest bacteria count in the gills, buccal cavity and skin as 1.48x10⁴, 1.56x10⁴ and 1.33x10⁴ cfu/ml respectively. The organism showed a positive reaction to gram test, citrate utilization test, catalase test, coagulase test and gives a negative reaction to motility and indole test. The result of the finding also showed that aqueous extract of garlic inhibited the growth of the organism at the concentrations of 125mg/l, 250mg/l and 500mg/l; bitter kola was only effective at 500mg/l. It was observed that onion had no inhibitory zone. Antibiotics such as Tetracycline Gentamycin, Nitofurantoin, Colistin, Streptomycin, Cotamozazole and Naladoxic acid were also used against the organism. Tetracycline, Gentamycin, Nitofurantoin, Colistin and Streptomycin had zone of inhibitions while Ampicillin, Cotamozazole and Naladoxic acid had no inhibition zones. Based on the result of this finding, garlic and bitter kola had antibacterial properties. Research work should be carry out in the development of new drugs with the use of garlic in fighting against micro-organism in pond water.

Keywords: Plant extracts, Staphylococcus aureus, Clarias gariepinus juvenile, Ogun, Lagos, Oyo states

INTRODUCTION
Aquaculture has been a growing activity for the last 20 years worldwide, and this impressive development has been attended by some practices potentially damaging to human and animal health (Naylor and Burke, 2005). The large scale settings of aquatic animal husbandry have resulted in an increased antibiotic resistance in bacteria potentially pathogenic to fish and related environment (Smith et al., 1994; Alderman and Hastings, 1998; Petersen et al., 2002; Alcaide et al., 2005; Cabello, 2006). The continuous use of antimicrobial agents in aquaculture has resulted in more resistant bacterial strains in the aquatic environment. Continuous use of synthetic antibiotics reveals the threats to consumers and non-target organism in the environment (Muniruzzaman and Chowdhury, 2004; Abutbul et al., 2005). Treatments of bacterial diseases with various plant extracts have been safely used widely in organic agriculture, veterinary and human medicine (Direkbusrakom, 2004). Since ancient
times, medicinal plants have been used for the treatment of common infectious diseases (Rios and Recio, 2005) and treatments with plants having antibacterial activity are a potentially beneficial alternative in aquaculture (Abutbul et al., 2005). Medicinal plants as alternative agents are effective in treating infectious diseases and mitigate many side effects that are associated with synthetic antimicrobials (Punitha et al., 2008). In addition, plant-derived phytomedicines provide a cheaper source for treatment and greater accuracy than chemotherapeutic agents (Punitha et al., 2008). Among the common fish pathogens, Escherichia coli and Pseudomonas spp are gram-negative, Staphylococcus aureus and Streptococcus agalactiae as gram-positive bacteria cause infectious diseases. A. hydrophila, the most common bacterial pathogen in freshwater fish, has been recognized to be the aetiological agent of several distinct pathological conditions including tail/fin rot and haemorrhagic septicaemia especially in freshwater and ornamental fish (Austin and Austin, 2007).

Staphylococcus aureus is a facultative anaerobic, Gram-positive coccus, and it is the most common cause of staphylococcus infections. It is frequently part of the flora found in the nose and on skin. About 20% of the human population are long-term carriers of Staphylococcus aureus. The carotenoid pigment staphyloxanthin is responsible for Staphylococcus aureus characteristic golden colour, which may be seen in colonies of the organism. This pigment acts as a virulence factor with an antioxidant action that helps the microbe evades death by reactive oxygen species used by the host immune system (Liu et al., 2005). Staphylococcus aureus can cause a wide range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, chest pain, bacteraemia, and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections (Bowersox, 1999). However, while the majority of Staphylococcus aureus are coagulase-positive, some may be atypical in that they do not produce coagulase (the most common organism in patients with nosocomial bacteraemia is coagulase-negative Staphylococcus). It can survive on animals such as dogs, cats, fish and horses, and can cause bumble foot in chickens. It can survive for hours to days, weeks, or even months on dry environmental surfaces depending on strain (Cimolai, 2008).

The ability of some herbs and seaweeds to inhibit activity of bacteria having potential interest as fish pathogens has been documented (Direkbusarakom, 2004; Muniruzzaman and Chowdhury, 2004; Abutbul et al., 2005; Borisutpeth et al., 2005; Dubber and Harder, 2008). Some of the local herbs and desert plants were reported to inhibit the pathogenic bacteria in aquaculture and referred to limited number of plant species (Direkbusarakom, 2004; Muniruzzaman and Chowdhury, 2004; Abutbul et al., 2005; Borisutpeth et al., 2005). Bacteria are important pathogens for both cultivated and wild fish, and are responsible for serious economic losses. Some bacteria cause only surface diseases as skin or gill infections, especially flexibacteria, but some inflict systemic disease (Inglis et al., 1993). The prevalent
fish diseases in fish farms are usually initiated by bacteria. Fish bacterial infections can occur as a bacterium, which implies the presence of bacterial organisms in the bloodstream without clinical signs. Others occur as a septicaemia, which indicates that bacteria and toxins are actually present in the circulatory system and usually precipitate disease and clinical signs. Inflammation, haemorrhage and necrosis are clinical signs associated with septicaemia. Gram-negative bacteria can produce either exotoxins or endotoxins, which consist of proteolytic enzymes that kill host cells and cause necrosis or can, make blood vessels more porous and cause haemorrhage (Kirjusina et al., 2007). Fish in husbandry are exposed to a multitude of stressors. Among the important stress-inducing factors are those with strong psychological components that cause fright, excitement and discomfort. Stress can be induced by such activities as handling, transport and weighing. Moreover, crowding at high densities also produces a variety of stimuli that cause stress (Petersen et al., 1998). Outbreaks of diseases are associated with depressed oxygen levels. Pre-disposing risk factors include also overcrowding, organic pollution and hypoxia (Kirjusina et al., 2007). In fish farms, mainly salmon farms, outbreaks are typically associated with stress, especially, high temperatures. The dominant fish bacterial diseases are furunculosis and myxobacteriosis of salmonids and aeromonosis of carps. Myxobacteriosis is a common bacterial disease that affects the skin or gills of freshwater fish. Flexibacter columnaris, the most prevalent member of this group, which has a worldwide distribution and can probably, infect most freshwater fish. It is an important fish pathogen and can rapidly infect a population and cause large mortalities (Kirjusina et al., 2007). Staphylococcus aureus, Pseudomonas spp, Escherichia coli are universally distributed and can be isolated from a wide range of environmental and water samples.

Antimicrobial drugs can either be antibiotics or chemical antimicrobials (chemotherapeutic agents). Antimicrobial substances produced by living micro-organisms are antibiotic. They can be culture extracts and filtrates of fungi such as penicillin and bacteria such as Bacillus spp. Antimicrobials act on bacteria in various ways including: inhibition of cell wall formation leading to cell lysis e.g. penicillin, changing the bacteria cell membrane, leading to loss of cell contents and to cell death e.g. polymyxins, inhibiting the production of nucleic acids, and therefore preventing bacteria from reproducing e.g. nalidixic and prevents DNA synthesis (Cheesbrough, 1984) as well as inhibiting protein production. Therefore arresting bacteria growth e.g. Tetracycline. The use of herbs and medicinal plants as the first medicines are a universal phenomenon. Every culture on earth, through written or oral tradition, has relied on the vast variety of natural chemistry found in healing plants for their therapeutic properties. Onion (Allium cepa) is one of the oldest cultivated vegetables in history. The relative pungency of onion has both genetic and environmental components. Sulphur compounds in onions have also been shown to be anti-inflammatory both by inhibiting formation of thromboxanes and by inhibiting the action of platelet-activating factor (PAF). Thiosulfinates condition anti-thrombotic benefits, including antioxidant activity (Ying, 1998), reduced serum cholesterol and enhance in vitro platelet activity (Goldman et al., 1995). This later effect is important for cardiovascular health by reducing the probability that platelets aggregate in the blood, a major cause of heart attacks and strokes (Havey, 1999). Hence, thiosulphinates
found in onion have been shown to inhibit in-vitro platelet aggregation (Ekwenye et al., 2005). Flavonoids are chemical compounds active against micro-organisms. They have been found in-vitro to be effective antimicrobial substance against a wide array of micro-organisms. Garlic (*Allium sativum*) is a bulbous, perennial and medicinal plant. It has inhibitory effect on many microbes (Banerjee and Maulik, 2002). The antimicrobial activity of this plant has been known for a long time. It is also claimed to help prevent heart diseases including atherosclerosis, high cholesterol, high blood pressure, and to improve the immune system as well as protection against cancer (Maryland, 2005). A daily dose of 1ml/kg body weight of garlic extract for six months can result in significant reduction in oxidant (free radical) stress in the blood of patients with atherosclerosis and cholesterol circulating in the bloodstream. Garlic’s ability to prevent these oxidation reactions may explain some of its beneficial effects in atherosclerotic cardiovascular diseases. *Garcinia kola* is used in folklore remedies for the treatment of ailments such as liver disorders, hepatitis, diarrhoea laryngitis, bronchitis and gonorrhoea (Iwu 1993; Adesina *et al*., 1995). The seed is masticatory and also used to prevent and relieve colic, chest colds, cough. It can as well be used to treat headache (Ayensu, 1998). Iwu (1993) reported the use of this plant for the treatment of jaundice, high fever, purgative and chewing stick. The plant also found usefulness in the treatment of stomach-ache and gastritis (Ajebesone and Aina, 2004). The phytochemical compounds isolated from *Garcinia kola* include oleoresin (Onayade *et al*., 1998), tannins, saponins, alkaloids, cardiac glycosides (Ebana *et al*., 1994). Other phytochemical compounds so far isolated from *Garcinia kola* seeds are biflavonoids such as kolaflavone and 2-hydroxybiflavonols (Okunji and Iwu, 1991; Terashima *et al*., 1999; Okunji *et al*., 2002). Two new chromanols, garcioic and garcinal, together with tocotrienol were reported isolated from *Garcinia kola* (Terashima *et al*., 2002). The biflavonones are predominant compounds in *Garcinia kola* and kola flavanones are major components of kolaviron (Iwu, 1993). *Garcinia kola* is used in folklore remedies for the treatment of various infections caused by pathogens.

**MATERIALS AND METHODS**

**Collection of Samples**

Samples were collected from fifteen private fish farms in Ogun, Oyo and Lagos states. Bacteria isolates from each fish samples were collected from the gill, skin and buccal cavity using swabbing method. The sterile cotton wool swabs were used to collect the samples. Each of the sterile cotton wool swab kit contains peptone water which serves as transport media. The swab kits were arranged and preserved in an ice box that contains block ice.

**Bacteriological Analysis**

1ml each of the original stock was suspended into 9ml sterile water aseptically in a MacCartney bottle which was then shaked together. Further dilution of $10^{-1}$, $10^{-2}$ and $10^{-3}$ were carried out, in which $10^{-2}$ dilution was later used. A loopful each of the stock culture was inoculated onto sterile Nutrient Agar plates. The Nutrient Agar plate was incubated at $37^{\circ}$C for 24 hours for bacterial growth. Three replicates were prepared for every sample examined.
Identification of Microorganism
The organisms were identified using Grams reaction and biochemical tests such as Catalase, Coagulase, Citrate Utilization, Urease, Nitrate Reduction, Indole Reaction, Oxidase, Sugar Fermentation tests and so on were carried out according to Akinyemi (2009) and Olutiola et al. (1991) to identify sample by their reaction to the tests.

Preparation of Cold Water Extract of Onion, Garlic and Bitter Kola
50g each of onion bulb, bitter kola and garlic bulb was weighed and mashed in a sterile mortar and pestle which was then soaked in 100ml of sterile water for 24 hours to extract the juice. After 24 hours, each of the extract was sieved by the use of muslin filter paper into different sterile universal bottle. Different concentrations (i.e. 500mg/ml, 250mg/ml and 125mg/ml) of each of the extract was gotten by taking 50ml each from the extract and diluting it with another prepared 100ml of sterile water to get the 250mg/ml. Also out of the 250mg/ml, another 50ml was also taken and diluted with 100ml of sterile water in another bottle to get the 125mg/ml. This procedure was used for the three plant extracts (onion bulb, bitter kola and garlic bulb).

Preparation of Standard Drugs for Comparism
Antibiotic discs were used as comparison for the antimicrobial activity with plant extracts. 8 antibiotics were used which includes; Ampicilin (25mg), Cotraimozazole (25mg), Gentamycin (10mg), Naildoxic acid (30mg), Naitrofurantoin (30mg), Colistin (25mg), Streptomycin(25mg), Tetracycline (25mg).

Determination of Antimicrobial Activity
2.8g of nutrient agar was dissolved in 1000ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000ml of distilled water in a conical flask. 30ml of the nutrient agar were poured into each MacCartney bottles and then sterilized in an autoclave at 160°C for 15minutes. After sterilization, the agar was allowed to cool and 1ml of $1 \times 10^5$ cfu/ml dilution of the organism was seeded into it. The agar were poured into sterile Petri dishes and allowed to set. A cork borer with diameter of 10mm were used to bore holes on the surface of the agar in 3 places into which 0.3ml of each of 500mg/ml, 250mg/ml and 125mg/ml of each of the extracts was poured. Also, the commercially available antibiotic disc was placed aseptically with the use of a forceps gently at the centre of another nutrient agar plate that has already been seeded with organism. It was then incubated at 37°C for 24 hours.

Determination of Minimal Inhibition Concentration
Cold water extracts of bitter kola (Garcinia kola), onion bulb (Allium cepa), and garlic (Allium sativum) were used for the minimum inhibitory concentration (MIC). Having obtained the different dilution and concentrations, three drops of overnight broth cultures of the test organism was inoculated into the different dilutions i.e. 500mg/ml, 250mg/ml and 125mg/ml and incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured with the aid of a transparent ruler and recorded.
Preparation of Standard Drugs for Comparison

Antibiotic discs were used as comparison for the antimicrobial activity with plant extracts. Eight (8) antibiotics were used which includes; Ampicilin (25mg), Cotraimozazole (25mg), Gentamycin (10mg), Naildoxic acid (30mg), Naitrofurantoin (30mg), Colistin (25mg), Streptomycin(25mg), Tetracycline (25mg).

RESULT

A total of 45 samples were collected and various tests were carried out to identify, and further test was performed to distinguish \textit{Staphylococcus aureus} from other microbes present. From table 1 below, it was observed that after the test has been carried out, the result showed that in all the fifteen farms, the organisms isolated gave a positive test for gram reaction, citrate utilization test, catalase test, coagulase test, and negative to motility test, indole test. The sugar fermentation test with glucose, sucrose and lactose was carried out. Table 2 showed the mean bacteria count recovered from the gill, mouth, and skin in all the farms as \(8.9 \times 10^3\), \(1.45 \times 10^4\), \(8.8 \times 10^3\), \(8.3 \times 10^2\), \(5.6 \times 10^3\), \(1.49 \times 10^4\), \(4.9 \times 10^3\), \(1.41 \times 10^4\), \(8.3 \times 10^3\), \(1.12 \times 10^4\), \(9.4 \times 10^3\), \(1.6 \times 10^3\), \(5.1 \times 10^2\), \(3.3 \times 10^2\), \(6.9 \times 10^3\) for Eweje, Abeokuta, Ultimate, Ikililu, Larry, Hope, Kulturetek, Durante, Zamit, Crams, Glorious, Feed vine, Palm royal, Quick link, Palm and wisdom respectively. In this study, \textit{Staphylococcus aureus} was recovered from the gills, buccal cavity and skin of juvenile \textit{Clarias gariepinus}. This shows that the bacteria count vary from each farm. From table 2, Unaab in Ogun state recorded the highest bacteria count in the gills, buccal cavity and skin as \(1.48 \times 10^4\), \(1.56 \times 10^4\) and \(1.33 \times 10^4\) respectively. Hope farm in Oyo state recorded the highest bacteria count in the skin region as \(2.89 \times 10^4\), Durante farm in Oyo state had the highest bacteria count in the buccal cavity, Quick link in Lagos had the lowest in the buccal cavity, and palm royal also in Lagos had no bacteria count in the buccal cavity. Quick link and feed vine both in Lagos had the lowest in the skin while palm wisdom had no bacteria count on the skin. Crams farm and Palm royal recorded the lowest bacteria count in the gills.

Generally the viable bacteria count of Ogun State and Oyo state were the highest while the bacteria count of Lagos state was relatively lower than the others. Table 3 showed the zone of inhibition when the organism was treated with plant extracts of garlic, onions and bitter kola at different concentrations of 500mg/l, 250mg/l, 125mg/l. The result showed that onion had no effect on the organisms from all the locations. It was observed that in all the locations, garlic had a very high effect on the organism and it was highest at 500mg/l. Bitter kola was only effective at a relatively low rate using 500mg/l while lower concentrations show no result. Table 4 showed that ampicillin, cotraimozazole, naladoxic acid all had no effect on the \textit{Staphylococcus aureus} while tetracycline, gentamycin, nitrofurantoin, colistin and streptomycin recorded zones of inhibitions at 18 mm, 19 mm, 22 mm, 17 mm, and 18 mm respectively.
**TABLE 1: Morphological, Biochemical and Identification of Bacteria Isolated from Clarias gariepinus from Farms across Ogun, Oyo and Lagos state**

<table>
<thead>
<tr>
<th>States</th>
<th>Farm</th>
<th>Gram reaction</th>
<th>Citrate utilization test</th>
<th>Catalase test</th>
<th>Coagulase test</th>
<th>Motility test</th>
<th>Indole test</th>
<th>Sugar fermentation test</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Glucose</td>
</tr>
<tr>
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<td>Eweje</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AG</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>Unaab</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>AG</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>Ultimate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AG</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>Ikiliki</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AG</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>Larry</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>AG</td>
<td>AG</td>
</tr>
<tr>
<td>Oyo</td>
<td>Hope</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>kulturetek</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>AG</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>Durante</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AG</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>Zamit</td>
<td>+</td>
<td>+</td>
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<td>-</td>
<td>-</td>
<td>AG</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>Glorious</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>AG</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>Feed vine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AG</td>
</tr>
<tr>
<td>Lagos</td>
<td>Palm royal</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>Quick link</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>Palm wisdom</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AG</td>
</tr>
</tbody>
</table>

**KEYS:** - negative, +positive, AG=acid and gas productivity

**Table 2: Bacteria Isolated From Fish Sample in cfu/ml**

<table>
<thead>
<tr>
<th>State</th>
<th>FARMS</th>
<th>Gill</th>
<th>Buccal cavity</th>
<th>Skin</th>
<th>MEAN</th>
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<tr>
<td>Ogun</td>
<td>Eweje</td>
<td>8.6×10³</td>
<td>9.3×10³</td>
<td>8.9×10³</td>
<td>8.9×10³±2.02</td>
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<tr>
<td></td>
<td>Abeokuta</td>
<td>1.48×10⁴</td>
<td>1.56×10⁴</td>
<td>1.33×10⁴</td>
<td>1.45×10⁴±6.74</td>
</tr>
<tr>
<td></td>
<td>Ultimate</td>
<td>6.4×10³</td>
<td>1.54×10⁴</td>
<td>4.6×10³</td>
<td>8.8×10³±3.34</td>
</tr>
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<td>Ikiliki</td>
<td>2.0×10²</td>
<td>1.4×10³</td>
<td>9.0×10²</td>
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<td>Larry</td>
<td>2.0×10³</td>
<td>8.0×10³</td>
<td>7.0×10³</td>
<td>5.6×10³±1.85</td>
</tr>
<tr>
<td>Oyo</td>
<td>Hope</td>
<td>8.4×10³</td>
<td>7.6×10³</td>
<td>2.89×10⁴</td>
<td>1.49×10⁴±6.97</td>
</tr>
<tr>
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<td>Kulturetek</td>
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<td>8.6×10³</td>
<td>2.8×10³</td>
<td>4.9×10³±1.85</td>
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<tr>
<td></td>
<td>Durante</td>
<td>1.8×10³</td>
<td>2.89×10⁰</td>
<td>1.16×10⁰</td>
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<tr>
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<td>Zamit</td>
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<td>4.3×10³</td>
<td>1.46×10⁰</td>
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<td></td>
<td>Crams</td>
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<td>5.2×10³</td>
<td>2.85×10⁰</td>
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<td>Lagos</td>
<td>Glorious</td>
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<td>8.6×10³</td>
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<td>Feed vine</td>
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<td>Quick link</td>
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<td>3.0×10²</td>
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<td>Palm wisdom</td>
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<td>7.1×10³</td>
<td>Nil</td>
<td>6.9×10³±1.50</td>
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TABLE 3: Result of Antimicrobial Susceptibility Test of Garlic, Onions and Bitter Kola on *Staphylococcus aureus* at different Concentrations (mg/l) Showing Zone of Inhibition

<table>
<thead>
<tr>
<th>States</th>
<th>Farms</th>
<th>Onions (mg/l)</th>
<th>Garlic (mg/l)</th>
<th>Bitter kola (mg/l)</th>
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<tr>
<td></td>
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<td>500 250 125</td>
<td>500 250 125</td>
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<tr>
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<td>Ultimate</td>
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<td>26 24 22</td>
<td>14 nil nil</td>
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<td>28 24 21</td>
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<td>16 nil nil</td>
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TABLE 4: Result OF Antimicrobial susceptibility Test of Antibiotics on *Staphylococcus aureus* using different MG (COKE BORER DIAMETER IS 10mm)

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<th>COT (25mg)</th>
<th>GEN (10mg)</th>
<th>NAL (30mg)</th>
<th>NIT (25mg)</th>
<th>COL (25mg)</th>
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Tet=tetracycline ,Amp=ampicillin ,Cot=cotramoazole ,Gen=gentamycin ,Nal=naladoxic acid, Nit=nitrofurantoin ,Col=colistin ,Str=streptomycin
DISCUSSION
This study was carried out with the purpose of assessing the inhibition of staphylococcus aureus by plant extracts and also to compare the inhibitory ability of the plant extracts with that of eight (8) selected conventional antibiotics. The incidence of microbes on the skin compared to other spots of reference is dependent on the interaction of the skin with the water; also increase in surface area can encourage the multiplicity of bacteria. According to the availability of the microbe on the skin, gills and buccal cavity, with respect to the result, we can deduce that despite the small size of the Clarias gariepinus juveniles the bacteria load was found higher in the skin. The result of effect of the plant extracts on the organism shows that at the three (3) tested levels (500, 250, and 125 mg/l), it was very active using garlic, while bitter kola was only active at 500mg/l. According to Banerjee and Maulik, (2002), the main antimicrobial constituent of garlic has been identified as the oxygenated sulphur compound, thio-2-propene-1-sulfinic acid S-allylester, which is referred to as allicin. Garlic had a very high inhibition due to the presence of allicin which is very active and also effective in the treatment of cancer. It also have many medicinal effects such as lowering of blood cholesterol level (Yeh and Yeh, 1994), anti platelet aggregation (Steiner et al., 1996), anti-inflammatory activity (Baek et al., 2001) and inhibition of cholesterol synthesis in man.

Out of the eight antibiotics used, nitrofurantoin produced the highest inhibition zone therefore, that it can be used at a considerable level against Staphylococcus aureus. Comparing the inhibitory ability of garlic to the effective antibiotics which shows that garlic was more effective, but the use of garlic and the most effective antibiotic (nitrofurantoin) may also be encouraged at a moderate rate in other to curb harmful effects on diseased fish. However, heavy antibiotics usage in aquaculture should not be encouraged and probably be replaced with alternative processes for treating fish diseases to avoid the emergence of antibiotic resistance in pathogenic and environmental bacteria (Sorum and L'Abée-Lund, 2002; Cabello, 2006). The herbal plants may be used as potential and promising source of pharmaceutical agents against fish pathogens in the organic aquaculture. The screening results of this study confirm the use of medicinal herbs as a source of antimicrobial agent for this purpose.

CONCLUSION
The study reveals that Staphylococcus aureus is present in the gills, skin and the buccal cavity of Clarias gariepinus juvenile fish. It was also concluded that this micro-organism tested positive to Grams Test, Citrate Test, Coagulase Test, while it also tested negative to Catalase Test, Motility Test, Indole Test and to the Test Sugar Fermentation processes it has been observed that Staphylococcus aureus produces both acid and sugar production. The result of present investigation emphasizes on the usefulness of garlic and bitter kola in the treatment of Staphylococcus aureus and need to enhance its exploitation, because it can be easily located in the local market and cost less when compared to the antibiotics used which may be readily available in the rural areas easily. This is most important to consider the multi-drug resistance strain of Staphylococcus aureus currently emerging worldwide (Prescott et al., 2005). Drugs of the past were substances with a particular therapeutic action
extracted from plants. More and more researchers find that food and their individual constituents perform similar fashion to modern drugs and sometimes better without the dreaded side effects (Wainright, 2001). Garlic is also better than most of the antibiotics used against intestinal disorder.

REFERENCES


