# PRELIMINARY STUDY ON THE ANTIBACTERIAL EFFECT OF ETHANOLIC EXTRACT OF VISCERAL ORGANS OF AFRICAN CATFISH *Clarias gariepinus* (BURCHELL, 1832) ON SELECTED BACTERIA

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Abstract: The use of raw visceral organs of fish for the treatment of wounds is common practice among local fishers in northern Nigeria. This preliminary study was carried out in order to scientifically assess the antimicrobial activities of visceral organs (VO) of *Clarias gariepinus* on some common Bacteria that were usually isolated from human wounds: *Staphylococcus aureus, Staphylococcus pyogenes, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*. The VO were dried, ground into powder and prepared at concentration of 300 and 500 mg/ml. The phytochemical (zoochemical) analyses were done. The antimicrobial sensitivity test was carried out using disc diffusion method in three replicates for each concentration per tested organism. The diameters of zones of inhibition (DZI) produced were measured with a ruler. The means of DZI from each of the three replicates were calculated and compared with those of standard antibiotics' DZI approved standard of Clinical Laboratory Standard International. DZI of 15.0 and 12.0 mm were the highest at concentrations of 500 and 300 mg/ml, respectively for *S. pyogenes* and at 500 mg/ml, there was DZI of 11.0 mm for *S. aureus*. The ethanol extracts contain Cardiac glycoside, Cardenolides, Terpenoid and Saponin. It was concluded that the extract has the potential to be developed as an antimicrobial agent against *S. pyogenes* at 500 mg/ml. Further research should be carried out with different extraction media (methanol. ethanol and aqueous) and test on organisms for antimicrobial activities at even concentrations higher than 500 mg/ml for the VO of the *C. gariepinus* and of other fish species.

**Keywords:** Antimicrobial agent, bacteria, *Clarias gariepinus*, ethanol extracts, visceral organs

## Introduction

The African catfish, *Clarias gariepinus* is remarkable and fascinating species as it is extremely hardy and withstands adverse environmental conditions. The fish can efficiently assimilate a wide variety of animal and plant protein. The predatory, cannibalistic and the voracious feeding habitat of *C. gariepinus* catch the attention of fish farmers to culture them in natural inland fresh water bodies (Dhawan and Kaur, 2001). It is tolerant to high water turbidity, low dissolved oxygen and often the last or only fish species found in remnant pools of drying river (Safrial and Brunton, 1991). *C. gariepinus* is considered to be omnivorous, displaying both scavenger and predator behaviour. It has also been observed that *C. gariepinus* feed on various fruit, seed, and aquatic invertebrates, small mammals and even planktons (Skelton, 2001).

Bacteria are often maligned as the causes of human and animal diseases. However, certain bacteria, the *Actinomycetes*, produce antibiotics such as streptomycin and nocardicin; others live symbiotically in the guts of animals (including humans) or elsewhere in their bodies, or on the roots of certain plants, converting nitrogen into a usable form. Bacteria are of such immense importance because of their extreme flexibility, capacity for rapid growth and reproduction (Blair *et al.*, 2015). Wounds are injuries in which layer(s) of skin is affected therefore, compromising the protective function of the skin. Kenneth (2017), reported wounds that were contaminated with various bacteria isolates identified as *Staphylococcus aureus* (62.7%), Coagulase negative *Staphylococcus* species (52.8%) and *Pseudomonas aeruginosa* (23.1%). Ogba *et al.* (2014) also reported *Staphylococcus aureus* (30.8%); *Pseudomonas aeruginosa* (17.3%); *Escherichia coli* (7.6%); *Proteus species* (8.1%); *Klebsiella pneumonia* (6.8%); *Citrobacter species* (9.2%); *Serratia species* (8.5%); *Coagulase negative Staphylococcus* (1.7%); *Streptococcus pyogenes* (1.0%); *Non-haemolytic Streptococcus* (0.3%); *Providentia species* (3.4%); *Morganella species* (1.7%) isolated from post-operative and non-operative wounds in studies of bacterial pathogens associated with wounds from 295 samples of patients.

It has been observed that some local fishermen use the wet or powder form of visceral organs from *C. gariepinus* and other fish species when wounded as a medicine through topical application and do claim to be healing their injuries. These practice and claim, as observed from our interactions with the fishermen, inspired this preliminary study in order to scientifically investigate the uses of visceral organs from *C. gariepinus* as antimicrobial agent and there is paucity of literature on such study. Panday (2012) reported that some plants and other animal species have ability to inhibit activity of bacteria due to some chemical compounds which they possessed such as Terpenoid, Alkaloid, Flavonoid and Tannins, Saponin.

Therefore, this study was aimed at determining the effect of visceral organs from the *C. gariepinus* as antimicrobial agent against some commonly isolated bacteria from human wounds.

## Materials and Methods

### *Sample collection*

The fish visceral organs were procured from Monday market in Maiduguri; the visceral organs were washed with 9% of saline solution. The weight obtained at the fresh stage was 500 g, then, it was sundried for about 3 – 4 days to remove the moisture contents; ground into powder and reweighed to be 80 g which was kept in air tight bottle before the experiment commenced.

### *Identification of the visceral organs*

The fish visceral organs were identified as kidneys, Lungs, Heart, Spleen and intestines at the Fish Biology Laboratory of the Department of Fisheries, University of Maiduguri (Plates 1 and 2).

**Plate 1: Some visceral organs of *Clarias gariepinus***

**Plate 2: Some visceral organs of *Clarias gariepinus***

***Ethanol extraction of powdered ground sundried visceral organs***

The ground visceral organs of *C. gariepinus* (fine powder) were subjected to extraction in the Chemistry Research Laboratory, Department of Pure and Applied Chemistry, University of Maiduguri. Five grams (5 g) of the dried powder of visceral organs of *C. gariepinus* was immersed in 250 ml of 96% of ethanol overnight. The extract was filtered using Whatman filter paper. The filtrate was concentrated to dryness under reduced pressure at tropical room temperature using 300 Mild Steel Oven dry. The extract was then transported to the Veterinary Research Laboratory of the Department of Veterinary Microbiology, University of Maiduguri for microbial sensitivity test.

***Phytochemical (Zoochemical) analyses***

The phytochemical (zoochemical) analyses were carried out with several tests at Chemistry Research Laboratory, University of Maiduguri using standard procedures described by Trease and Evan (1989) and Sofowara (1993).

***Preparation of ethanol extracts of powdered visceral organs of C. gariepinus***

The powdered visceral organ (concentrated filtrate to dryness) of *C. gariepinus* organs were prepared by dissolving 0.3 and 0.5 g in 1 ml of ethanol which also gave concentration of 300 and 500 mg/ml, respectively.

***Collection and identification of some pure culture bacteria isolated from wounds***

Some bacteria commonly found in literature that are usually isolated from wounds are *Staphylococcus aureus*, *Staphylococcus pyogenes* and *Bacillus subtilis*. The pure cultured of these bacteria were obtained from the Veterinary Research Laboratory of the Department of Veterinary Microbiology, University of Maiduguri. The samples obtained were used for the antimicrobial sensitivity test.

***Antimicrobial sensitivity test and experimental design***

The Antimicrobial Sensitivity Test was carried out using disc diffusion method describe by the Clinical Laboratory Standards (CLSI, 2014a). The standardized overnight culture of each bacterium containing approximately, $10^6$ cfu/ml was replicated three times for each concentration of ethanol extracts to be applied. The Petri dishes' lids were in place while the plates were inoculated at 37$^o$C overnight. The antimicrobial extracts were added to the inoculated plates at concentrations of 300 and 500 mg/ml of the extracts in separate petri dishes. The plates were inverted and allowed to stand for 30 min for the extracts to diffuse into the agar after which the plate was incubated aerobically at 35$^o$C overnight. The diameter of the zones of inhibition produced by each antimicrobial disc was measured with a ruler in millimetres and duly recorded for each bacterium and concentration of ethanol extract.

The in-vitro susceptibility test of Staphylococci by disc diffusion method is displayed in Table 1.

**Table 1: In vitro susceptibility test of Staphylococci by disc diffusion method**

| Antibiotics | Drug concentration (mg/ml) | Zone Diameter Bank point nearest to whole number (mm) | | |
|---|---|---|---|---|
| | | S | I | R |
| Gentamycin | 10 | ≥15 | 13 – 14 | ≤12 |
| Tetracycline | 30 | ≥19 | 13 – 18 | ≤12 |
| Oxacilline | 1 | ≥13 | 11 – 12 | ≤10 |

S = Susceptibility; I = Intermediate; R = Resistant

**Source:** Clinical Laboratory Standard International (CLSI, 2014b)

***Statistical analysis***

The data were subjected to descriptive analysis by expressing the diameters of zones of inhibition from each of the three replicates of ethanol extracts of the visceral organs from *C. gariepinus* per concentration per bacteria species as means. The means of the zones of inhibitions were compared with those of standard antibiotics' zones of inhibition for antimicrobial disc susceptibility testing approved standard of Clinical Laboratory Standard International (CLSI, 2014b).

**Results and Discussion**

Table 2 showed the results of Phytochemical screening of ethanol extracts of the visceral organs (fine powder) from *C. gariepinus*. The evaluation of the phytochemical indicates that, it contained, Cardiac glycoside, Cardenolides, Terpenoid and Saponin. However, Alkaloid, flavonoid, Phlabotannins, Tannin and Carbohydrates were absent in the extracts. The findings in this study have confirmed the claims by folklore medicine that some plants and animals waste were used for the treatment of infections such as dental caries, pneumonia, wounds, otitis, dermatosis, anti-inflammatory and antibacterial activity (Musa *et al.*, 2008). Several other studies have reported similar phytochemical compounds from plants and or closely related species. These chemical compounds are the secondary metabolites that are known to be biologically active and may be contributing to the antimicrobial activity (Tsuchiya *et al.*, 1996). Flavonoid exhibit wide range of biological activity which includes antimicrobial and anti-inflammatory properties and it contain flavan-3-ol (flavonoids) compound which also possesses antibacterial activity (Zhang *et al.*, 2013). The mechanisms of antibacterial activity of flavonoids had been reported to be as a result of their ability to form complexes with bacterial cell walls (Musa *et al.*, 2008). The antibacterial activity of the visceral organs' ethanol extracts in this study was supported by Tamil *et al.* (2011) who reported that saponin component such as olean-12-on-28-oic acid (aridanin) possesses antibacterial property with their mode of action attributed to their ability to cause cell membrane lysis leading a leaking of protein and certain enzymes from bacterial cell.

**Table 2: Phytochemical (Zoochemical) screening of ethanol extracts of powdered viscera organs from *Clarias gariepinus***

| Chemical compound test | Result |
|---|---|
| Alkaloid | - |
| Flavonoid | - |
| Cardiac glycoside | + |
| Cardenolides | + |
| Terpenoid | + |
| Saponin | + |
| Carbohydrate | - |
| Phlabotannins | - |
| Tannins | - |

**+ = Present; – = Absent**

**Table 3: Means of zones of inhibitions from the triplicates of ethanol extracts of powdered viscera organs from *C. gariepinus* as antimicrobial agent against some bacteria causing infections in wounds**

| Concentration/ Cultured bacteria | 500 mg/ml Diameter Zone of Inhibition (mm) | 300 mg/ml Diameter Zone of Inhibition (mm) |
|---|---|---|
| *S. aureus* | 11.0 | 0.0 |
| *S. pyogenes* | 15.0 | 12.0 |
| *B. subtilis* | 0.0 | 0.0 |
| *E. coli* | 0.0 | 0.0 |
| *P. aeruginosa* | 0.0 | 0.0 |

Tables 3 represented the results of antimicrobial activity of visceral organs (fine powder) of *C. gariepinus* ethanol extract on some cultured bacteria (*S. aureus, S. pyogenes, B. subtilis, E. coli* and *P. aeruginosa*) that are usually causing infections in wounds. The diameter of zones of inhibition (DZI) of 15.0 and 12.0 mm were the highest at concentrations of 500 and 300 mg/ml respectively for *S. pyogenes*. Also, at concentration of 500 mg/ml there was DZI of 11.0 mm for *S. aureus*. However, there was no any DZI observed for *B. subtilis, E. coli* and *P. aeruginosa* at both concentrations of 500 and 300 mg/ml. The results of crude extract of visceral organs of *C. gariepinus* ethanol extracts showed concentration dependent pattern with antimicrobial activity on some selected bacteria. The results of the present study indicated that two out of the five test organisms were susceptible to the crude extract of visceral organs.

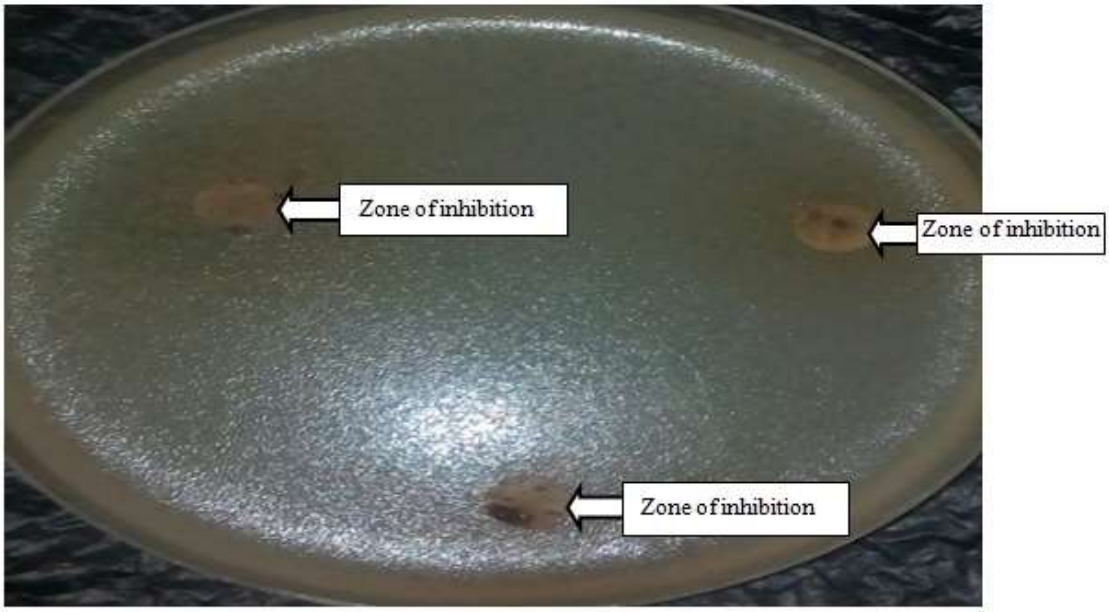


**Plate 3: Disc diffusion test of ethanol extracts of visceral organs from *C. gariepinus* on *S. aureus,* showing various zones of inhibition**

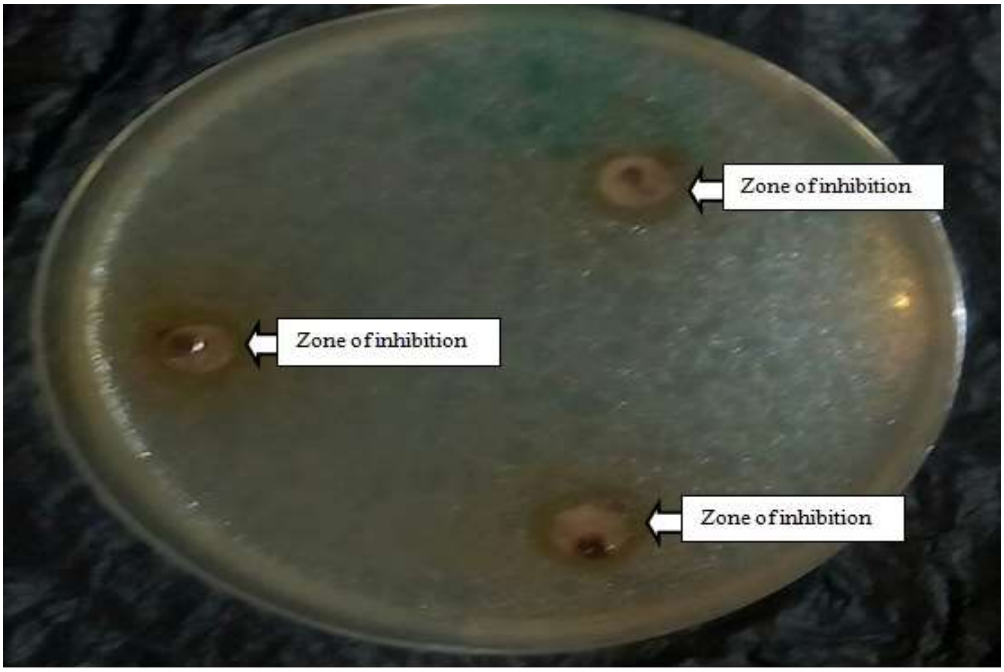


**Plate 4: Disc diffusion test of ethanol extracts of visceral organs from *C. gariepinus* on *S. pyogenes*, showing various zones of inhibition**

The DZI of 15.0 mm was comparable to Gentamycin susceptibility range of antimicrobial activity as reported by CLSI (2014b) while 12.0 and 11.0 mm were within the range of antimicrobial activities of Oxacilline recommended by CLSI (2014b). From these, it could be inferred that the ethanol extracts were bactericidal at 500 mg/ml. However, the antibacterial activity observed in this study could be associated with some of the secondary metabolites detected in the ethanol extracts of visceral organs (fine powder) from *C. gariepinus*. The samples of the disc diffusion test are in Plates 3 and 4.

**Conclusion**

Based on the findings of this preliminary study, it can be concluded that the results recorded from the antibacterial activity of the ethanol extracts of powdered visceral organs of *C. gariepinus* has the potential to be developed as an antimicrobial agent for the control of wound infected by *Staphylococcus pyogenes* at 500mg/ml. It is also concluded that the ethanol extracts contain Cardiac glycoside, Cardenolides, Terpenoid and Saponin which are phytochemical compounds that are known to possess antibacterial properties.

**Recommendation**

From the results of the study, it is recommended that further research should be carried out with different extraction media (methanol. ethanol and aqueous) and test organisms for antimicrobial activities at varying concentration levels even at concentrations higher than 500mg/ml for the visceral organs of the *C. gariepinus* and those of other fish species.

**Conflict of Interest**

Authors declare that there is no conflict of interest reported in this work.

**References**

Blair JMA, Webber MA, Baylay AJ, Ogbolu DO & Piddock LJV 2015 Molecular mechanisms of antibiotic resistance. *Natural Review Microbiology* 13: 42–51.

CLSI 2014a. *Methods for Broth Dilution Susceptibility Testing of Bacteria Isolated from Aquatic Animals;* approved guideline VET04–A2. Clinical and Laboratory Standards Institute. Wayne, Pennsylvania.

CLSI 2014b. *Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated from Aquatic Animals;* second informational supplement. Clinical and Laboratory Standards Institute (CLSI) Document VET03-VET04-S2. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania.

Dahiru D, Ezeaggwu SMC & Williams ET 2004. Antivity of the extracts of *Guiera senegalensis* and *Ziziphus mauritiania* on *Samonella gallinarium* and *Salmonella pollorum*. *Sahel Journal Veterinary Science*, 3: 7-9.

Dhawan A & Kaur K 2001. C hines gariepinus in Punjab water, Fishing Chines. *Journal of Fishing Methods,* 56: 72-80.

Kenneth IEP 2017. Identification of bacteria associated with wounds in Wukari and Environs, North-East, Nigeria. *AASCIT Journal of Health,* 4(5): 63 – 67.

Musa AM, Abbas G, Aliyu AB, Abdullahi MD & Akpulus IN 2008. Phytochemical and antibacterial screening. Of indigofera conferta Gillert (Papilioneceae). *Res. J. Medic. Plants* 2(2):74-78.

Ogba OM, Olorode OA, Adie GP 2014. Bacterial pathogens associated with wound infections in Calabar, Nigeria. *Mary Slessor J. Med.*, 13(1): 26 – 33.

Panday 2012 Mechanisms of bacterial biocide and antibiotic WSX5TXZGVF resistance. *J. Appl. Microbio.*, 92: 55–64.

Safrial O & Brunton MN 1991. A Co-operative Aquaculture Researched Programmed for South African National Scientific Programmed Report 89. CSIR, Pretoria, 79pp.

Skelton P 2001. A Complete Guide to the Fresh Water Fishes of South Africa Stunk Publishers. Cape Town, pp. 96-99.

Sofowora A 1993. Medicinal Plant and Traditional Medicinal in Africa. Spectrum Books Limited, Ibadan, Nigeria, p. 289.

Tamil SA, Dinesh MG, Satyan RS, Chandrasekaran B & Rose C 2011. Leaf and seed extracts of *Bixa orellana* L. exert anti-microbia;l activity against bacterial pathogens. *J. Appl. Pharmac. Sci*., 1(9): 116-120.

Trease GE & Evan WC 1989. Textbook of *phamcogenesy*, 14 Edition. W.B Salmelas company Limited. 24-29 Oval, Landon. N.W. 7 DX, UK and printed by Harcourt Brace and company. Asiapte by led 588. Orchard Road number 09-01 Form Singapor 238884, pp. 13-53, 112-139, 227-293, 334, 471-551.

Tsuchiya H, Sato M, Miyaxaki T, Fuji-wara S, Tanigaki S, Ohyama M, Tanaka T & Hinuma M 1996. Compative study on the antibacterial activity of phytochemical flavonones against methicillinresistant *Staphyloccus aureous. Journal of Ethnopharmacology*, 50: 27 – 34.

Zhang G, Fan D, Xing B, Kong T & Zhu X 2013. Isolation of new flavan-3-ol and ligand glycodsite from loropetalum chinense and their antimicrobial activities. *Fitotepia*, 90: 32-228.