Phytochemical Screening and Antibiogram of Ethanolic extracts of *Costus afer* stem on Microorganisms isolated from Fresh *siluriformes*

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***Abstract*: The Phytochemical and antibiogram of Costus afer stem on microorganisms isolated from the skin and other body part of fresh Siluriformes was studied using standard (analytical procedures). Different sample points of the fresh Silurformes were accessed microbiologically (Abdomen, Back Tail and Fins). The abdomen had the bacterial count which ranged from 5.4 x 10^4cfu/g to 7.2 x 10^4cfu/g and fungal count which ranged from 0.5 x 10^4cfu/g to 1.1 x 10^4cfu/g. The fins had the bacterial counts of 1.2 x 10^4 cfu/g to 5.5 x 10^4cfu/g and the fungi ranged from 0.2 x 10^3cfu/g to 1.2 x 10^4cfu/g. The bacterial isolates identified and their percentage frequencies of occurrence were Pseudomonas sp (36.34%) as the highest, Micrococcus sp (23.41%), Streptococus sp (20.49%), Staphylococcus sp (18.05%) and Proteus sp(11.71%) and the least was Proteus sp (11.71%). The fungal genera identified and their percentage frequencies of occurrence were Aspergillus sp (42.86%), Penicillium sp (20.41%), Mucor sp (25.51%) and Rhodotorula sp (11.22%). The phytochemical and qualitative analyses of Costus afer with ethanol reveal the presence of Alkaloids (0.614), Flavonoids (0.401), Saponins (3.003), Tannins (0.971) and Cyanogenic glycosides (0.764). Organisms like Staphylococcus sp and Micrococcus sp were susceptible to the aqueous extracts at 200% concentrations while the grams negative organisms were highly resistant to the ethanolic extract at all concentration. From the result, Costus afer can be used to sterilize fresh Siluriformes during preparation as it is capable of reducing the level of some microorganisms and enhancing the safety of the fish delicacy for consumption.**

***Keywords*: Photochemical, *costus afer*, *siluriformes*, microorganisms, ethanolic extract.**

I. INTRODUCTION

Costus afer is a tall perennial semi-woody herb with leafy canes measuring up to 3 meters high and bearing terminal inflorescences of white and yellow flowers. It is commonly found in moist places of West and tropical Africa and it is often planted in home gardens for medicinal purposes (Iwu, 2006).

Costus afer belongs to the family Zingiberaceae, it is a monocot and unbranched tropical plant with creeping rhizome. In Nigeria, it is known as Ukhueruoha in Edo, Mbritem in Efik, and Mbiritem in Ibibio. *Costus afer* is commonly called bush sugar cane or monkey sugar cane (Nyananyo, 2006).

Costus afer is found in tropical areas and comprises about 70 species, of which about 40 species are found in America, about 25 in Africa and about 5 in South-East Asia (Aweke, 2007).

Costus afer is a useful medicinal plant that is highly valueable for its anti-diabetic, anti-inflammatory and anti-arthritic properties in South East and South-West Nigeria. Among the Ikwere ethnic group in River State, it is applied in various ways. The leaves are reputed to be an effective remedy for fever and malaria when boiled with leave of *Carica papaya* (pawpaw) Citrus species (orange) and the bark of *Mangfera indica* (mango). The stem and juice have been used traditionally for treatment of cough, and measles. The juice of *Costus afer* is extracted and used as an instillation for eye inflammation and defects. The young and tender leaves when chewed are believed to give strength to the weak and dehydrating patient especially pregnant women.

In West Africa *Costus afer* is sometimes cultivated for the stem bark which is used to make mats and baskets it is also used as fodder for small ruminants poultry and the leaves serves as feed for snails and it is widely used for ceremonial and religious purposes. (Hamilton, 2003). *Silurformes* are group of bottom- feeding fish found in fresh water habitats and coastal regions and around every continent in the world except of Antarctica.

Silurformes are most easily identified by their flattened broad heads and the long whisker-like barbells that protrude from the month of the *Silurformes* (Gram & Huss, 2010).

The long barbells of the *Silurformes* contain the taste buds of the *Silurformes* and so are often most commonly used for smelling and therefore sensing what is about to eat (and to hide from) in the surrounding waters.

Phytochemical (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provides health benefits for humans rather than those attributed to macronutrient and micronutrient (Hasler and Blumbera, 2014). Since they have been difficulty in the washing and sterilizing of fresh water to remove microbial load, this work thus aims at using *costus afer* stem juice:-

- To isolate and analyzed organism from the different body parts of a fresh *Silurformes*.
- To carry out phytochemical analysis of the leaf *Costus afer*
- To carry out susceptibility testing *costus afer* stem extract on the isolated organism.

II. MATERIAL AND METHODS

Sample Collection Costus afer

The 5 fresh *Silurformes* sample used was bought from Itu market in Akwa Ibom State into a sterilized polythene bag. *Costus afer* was obtained from Ikot Osurua community into a sterile polythene bag. All samples were transferred to Department of Science Technology for further analysis. *Costus afer* was subjected to identification and characterization by a botanist in the biology unit, Science Technology and later transferred to Chemistry Laboratory for phytochemical analysis, while the fresh *Silurformes* sample was transferred to microbiology laboratory for microbiological analysis, and isolated were sampled aseptically from different body parts of the fish which included abdomen, back, tail and fins.

Bacteriological Analysis of the Sample

The fresh *Silurformes* used was caught into different pieces according to their body parts and was pounded with a sterile mortar and pestle. One (1g) of the pounded fresh *Silurformes* sample was introduced into a sterile 250ml beaker containing 9m1 of distilled water and mix properly. The beaker was corked with cotton wool and allowed to stand for 15minutes. A ten folds serial dilution of the fresh *Silurformes* sample was carried out. About 1m of the diluents from the dilution factor 10^2 and 10^4 was poured into sterile Petri dishes with an already prepared nutrient agar and MacConkey agar respectively. The plates were inoculated and incubated inverted for 18 hours before observing for microbial growth and at room temperature for 4-5 days for fungal growth.

Enumeration of Bacteria Isolates

The emerging visible discrete colonies in the inoculated plate were counted and expressed in colony forming unit per gram (cfμg). Colony appearances for all different growths in each plate were carried out in order to identify their growth characteristics such as shape, size, elevation, pigmentation, edge and appearance.

Sub culturing of all the grown colonies were done by streak method on freshly prepared agar plate. These were incubated for 24 hours at 37°C and pure colonies transferred to sterile McCartney bottle with prepared fresh agar slant. It was incubated at 37°C for 24hours and than stored in refrigerator at 4°C for further biochemical analysis as describe by Cheesbrough, (2006).

Identification and Characterization of Bacterial Isolates

The isolates were identified using their colonial appearance and also following the method of (Cheesbrough 2006). Gram and spore staining were done and the following biochemical test were carried out catalase test, coagulase test, citrate test, urease test, oxidase test, motility test, indole, methyl red and vogos proskauar (MRVP) and carbohydrate fermentation test as presented in appendix 1.

Fungal Analysis

Macro-Examination of Fungal Isolates

After 4 days of incubation at room temperature, the fungal culture plate was observed for visible growth. The surface colour and appearance of the growth were observed as well as the reverse colour of the isolate on the plate.

Micro-Examination of Fungal Isolates

The fungal isolates were examined by wet preparation method using Lactophenol blue indicator on a clean grease free glass slide and examined with x40 objective for identification of microscopic features (Collins and Lynes, 2004).

Antibiogram Analysis

Costus afer Extraction

The stems of *Costus afer* were properly cut into pieces with a sterile knife and were washed with distilled water. It was then pounded with a sterile mortar and pestle. About 200g of the pounded stems was weighed into a flat bottom flask containing 200m1 of 75% ethanol and aqueous solution, it was shaken constantly at an interval of 20 minutes for 72 hours (3 days) for proper extraction while aqueous extraction was carryout for 24 hours.

The set- up was filtered using sack cloth (sterile white handkerchief) and the filtrate was transferred into a beaker and placed in a water bath regulated at temperature of 50°C for dryness through evaporation method. The dried extract (paste was carefully transferred into a sample bottle. Sealed immediately and stored for further analysis in a refrigerator at 4°C.

Preparation of Antibiotic Sensitivity Disc.

Using a paper perforator, an absorbent Whatman filter paper was perforated at 6mm diameter and sterilized in a hot air oven at 160°C. Different concentration of (1/100 ½ ¼ 1/8 and 1/16) of *Costus afer* stem extract (paste) was obtained from the ethanol and aqueous extraction of leaves samples. The prepared disc were soaked into the different concentrations of extracts and was dried in an hot air oven at lower heat intensity for proper impregnation and then stored in a sterile MarCarkney bottle for further use.

Sensitivity Test

Sterile Muller Hilton agar were prepared and allowed to solidify and surface dried. Using a sterile inoculating loop,

each test organism was aseptically emulsified in physiological saline, and allowed to stand for 30 minutes for proper emulsification. Using a sterile swab stick the emulsified tested organisms were inoculated uniformly to cover the entire surface using streaking method and the excess was drained off using the disc diffusion techniques (DDT). A sterilized forceps was used to transfer the prepared ethanol and aqueous sensitivity disc to five different positions, on the plate and labeled properly based on their concentration and incubated inverted at 37°C for 24 hours. Observation of zone of inhibition around the disc on plates was measured with ruler in millimeters (mm) and the level of sensitivity-/-resistance of the organism to the extract was measured with a control antibiotic (Streptomycin).

Phytochemical Analysis

Determination of Alkaloids

About 5g of the dried grinded leaves sample was measured into a beaker containing 50ml of 10% ethanol + acetic acid. The mixture was well shaken and allowed to stand for 4 hours before filtering. TI was evaporated to one quarter of its original volume. A drop wise concentration of ammonium hydroxide solution was added. The precipitate obtained was oven dried for 30 minutes at 60°C and reweighed. The alkaloid content of the sample was determined using the equation:

$$\text{Percentage alkaloid} = \frac{W_2 - W_1}{W} \times \frac{100}{1}$$

Where W = weight of sample

W_1= Weight of empty crucible

W_2 = weight of paper + precipitate

Determination of Tannins

About 2g of dried grinded leaves sample was measured into a beaker, and 50m1 of distilled water was added and the beaker shaken. The mixture was allowed to stand for 30minutes at 28°C before it was filtered through Whitman No. 42 grade of filter paper. 2mls of the filtrate was dispensed into a 500mls volumetric flask. 2mls standard tannin solution (tannin acid) and 2mls of distilled water were measured into separate volumetric flask to serve as standard and the reagent was added to each of the flask and 2.5m1 of standard Na_2CO_3 solution was added. The content of each was made up to 50m1 with distilled water and allowed to incubate at 20°C for 90 minutes. Their respective absorbance was measured in a spectrophotometer at 560nm.

Determination of Saponins

Two grams (2g) of the sample was placed in a .beaker and 5Omls aqueous solvent (20% ethanol) added. The mixture was incubated with periodic agitation in a water bath at 55°C for 3 hours. This was filtered and the residue was re-extracted with another 100ml of the ethanol; Both extract obtained was mixed together and reduced by evaporating to about 40m1. Thereafter the concentrate was transferred to a 250m1 separating funnel and equal volume of diethyl ether added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer recovered. Re- extraction by partition was done repeatedly until the aqueous layer was clear in colour. The pH was adjusted to 4.5 using dilute NaOH in drops. The saponin was extracted finally with successive portion of 60ml and 10ml of N-butanol was added to it. The combine extract was washed twice in 10ml of 5% aqueous sodium chloride (NaCI) solution and evaporated to dryness in a pre-weighed beaker (W_1) in an oven at 60°C and reweighed (W_2). The saponin content was calculated in F.

$$\text{Saponin \%} = \frac{W_2 - W_1}{W} \times \frac{100}{1}$$

Where; W = weight of sample

W_1= Weight of empty crucible

W_2 = weight of paper + precipitate.

Determination of Flavoniod

10g of the plant sample was extracted repeatedly in 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No.42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over water and weighed to a constant weight.

Determination of Cyanogenic Glycoside

This is done using titrimetric methods of Vogel, (2002). About 1g of the sample was dissolved in 50m1 of distilled water in a corked conical flask and was allowed to stay overnight. The solution was filtered and the filtrate was used for the cyanide determination. 1ml of the filtrate was placed in a corked test tube; 4m1 of alkaline picrate solution was added and incubated in a water bath for 5minutes. After colour development the absorbance was read using spectrophotometer at 490mm. the absorbance of the blank containing only 1ml of distilled water and 4m1 alkaline picrate solution was also read. The cyanide contents were determined by extrapolation from the standard curve and the concentration calculated using the formula.

$$\% = \frac{\text{Absorbance of test solution}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{\text{weight of sample}} \times \frac{100}{1}$$

III. RESULTS AND DISCUSSION

Results

The phytochemical and antibiogram of *Costus afer* stem juice extract on microorganisms isolated from the skin and other body parts of fresh *Silurformes* was studied using standard analytical procedures. The results obtained from this research study are presented in tables.

Table 4.1: Shows the total bacterial count from the different sample points of fresh *Siluriformes*. The microbiological analysis of sample points of fresh *Silurformes* such as abdomen, back, tails and fins were also studied. In abdomen, the bacterial count range from 5.4 x 10^4 cfu/g to 7.2 x cfu/g, the back range from 3.6 x 10^4 cfu/g to 8.1 x cfu/g, the tail range from 3.7 x 10cfu/g to 5.2 x 10cfu/g and the fins range from 4.6 x 104 cfu/g to 11.2 x 10^4 cfu/g.

Table 4.2: Shows the percentage frequency occurrence of the bacterial genera isolated from the different sample points of fresh *Siluriformes*. From the table, the highest percentage frequency of occurrence among bacteria was observed from *Pseudomonas* sp. 26.34% followed by *Micrococcus* sp 23.4 1%, *Streptococcus sp*. 20.49%, and *Staphylococcus sp*. 18.05% and the least observed was *Proteus sp* 11.71%.

Table 4.3: Shows morphological and biochemical characteristic of the bacteria genera isolated from the different sample points of fresh *Silurformes*. From the table, the bacteria genera identified were *Pseudomonas* sp, *Micrococcus* sp, *Streptococcus* sp, *Staphylococcus sp* and *Proteus* sp.

Table 4.4: Reveals the total fungal count isolated from the sample points. From the table, in abdomen the fungal counts range from 0.5 x 10^3 cfu/g to 1.1 x cfu/g, the back range from 0.6 x 10^3 cfu/g to 1.3 x 1 cfu/g, the tail range from 0.4 x 1 cfu/g to 1.7 x 1 cfu/g and the fins also range from 0.2 x 103 cfu/g to 1.2 x 10 cfu/g.

Table 4.5: Shows the percentage frequency occurrence of the fungal isolates. From the table, the highest percentage frequency of occurrence was observed for *Aspergillus sp* with 42.86% followed by *Penicillium sp* 20.4 1%, *Mucor sp* 25.5 1% and the least being *Rhodotorula sp* with 11.22%.

Table 4.6: Shows the cultural and microscopic examination of the fungal genera. From this research study the fungal isolates identified are *Aspergillus sp*, *Penicillium sp*, *Mucor sp* and *Rhodotorula sp*.

Table 4.7: Shows the phytochemical compositions of the ethanolic extract of *Costus afer* stem to include alkaloids, flavonoid, saponins, tannin and cyanogenic glycosides. Qualitative analysis revealed moderately concentration (++) in saponins, tannins, and cyanogenic glycosides and scanty concentrations (+) in only flavonoids while alkaloids was not detected (absent). The quantitative analysis recorded the highest concentration in saponins (5.272) followed by tannin (2.380) and cyanogenic glycoside (0.958), flavonoids had (0.611) while alkaoids recorded the least concentration (0.320).

Table 4.8: Shows the phytochemical compositions of the aqueous extract of *Costus afer* stem to include alkaloids, flavonoid, saponins, tannin and cyanogenic glycosides. Qualitative analysis revealed scanty concentration (+) in alkaloids, saponins and tannins, and moderate concentration in flavonoid, while cyanogenic glucosides is absent. The quantitative analysis recorded the highest concentration in saponins (3.003), flavonoid,(1.401), followed by tannin (0.971), cyanogenic glycoside (0.764), while alkaloids recorded the least concentration (0.614).

Table 4.9: Shows the antibiogram of ethanolic extract on the bacteria isolates. From the table the Gram negative bacteria isolate were highly resistance to all concentrations while *Staphylococcus* sp and *Micrococcus* sp varied in their susceptibility.

Table 4.10: Reveals the antibiogram of aqueous extract on the bacterial isolates. From the study, *Pseudomonas sp*, *Proteus sp* and *Streptococcus* sp were highly resistance to all concentrations while *Staphylococcus sp* and *Micrococcus* sp were sensitive at the 200% concentration only as present on table 4.1.9 and 10 respectively.

Table 4.1: The Mean Total Bacterial Count Isolated from Different Sample Points of Fresh *Silurformes*

Sample Points	Specimen	No of Colonies/TBC cfu/g
Abdomen	Cf 1	6.3 x 10^4
	Cf 3	5.4 x 10^4
	Cf 5	7.2 x 10^4
Back	Cf 1	5.7 x 10^4
	Cf 2	3.6 x 10^4
	Cf 5	8.1 x 10^4
Tail	Cf 2	3.7 x 10^4
	Cf 3	4.8 x 10^4
	Cf 4	5.2 x 10^4
Fins	Cf 1	5.5 x 10^4
	Cf 3	4.6 x 10^4
	Cf 5	11.2 x 10^4

Keys
CF — Siluriformes
TBC- Total Bacterial Count

Table 4.2: The Percentage Frequency Occurrence of the Bacterial Genera Isolated from the Different Sample Points of Fresh *Siluriformes*.

Bacterial Isolate	Frequency of Occurrence (n-205)	Percentage Frequency of Occurrence (%)
Pseudononas sp	54	26.34%
Micrococcus sp	48	23.41%
Streptococcus sp	42	20.49%
Staphylococcus sp	37	18.05%
Proteus sp	24	11.71%
Total	**205**	**100%**

Table 4.3: Summary of biochemical analysis of isolates from different sample points of fresh *Siluriformes*

Isolates	1	2	3	4	5
Morphological characteristics	Rhizoid irregular greenish opaque	Circular roundish reddish translucent	Punctiform flat whitish transparent	Spirdle flat yellow dull opaque	Irregular lumbroid dull shiny opaque
Cell shape	Long rods	Scattered cocci	Cocci in chains	Cocci in cluster	Rods
Grams reaction	-	+	+	+	-
Latalase	+	-	-	+	+
Coagluase	-	+	-	-	+
Oxidase	+	-	-	-	+
Citrate	+	-	-	-	-
Urease	+	-	-	+	+
Motility	-	-	-	-	+
MR	+	-	-	-	+
VP	+	-	-	-	-
Sugars Test					
Lact	AG	AG	AO	AG	OO
Suc	AO	AO	AG	AG	OO
Glu	AO	AO	AG	AG	AG
mann	AO	AG	AG	AG	OO
Probable organism	*Pseudonas sp*	*Microococcus sp*	*Streptococcus sp*	*Staphylococcus sp*	*Proteus sp*

Table 4.4: The Total Fungal Count Isolated from the Different Sample Points of Fresh *Siluriformes*

Sample points	Specimen	Total Fungal Count of Colonies/TFC Cfu/g
Abdomen	Cf 1	1.1×10^4
	Cf 3	0.5×10^3
	Cf 5	1.0×10^4
Back	Cf 1	0.6×10^3
	Cf 2	1.3×10^4
	Cf 5	0.9×10^3
Tail	Cf 2	0.9×10^3
	Cf 3	1.7×10^4
	Cf 4	1.4×10^4
Fins	Cf 1	1.2×10^4
	Cf 3	- - - -
	Cf 5	0.2×10^3

Keys
CF – *Siluriformes*
TFC- Total Fungal Count

Table 4.5: The Percentage Frequency Occurrence of the Fungal Genera Isolated from the Different sample Points of Fresh *Siluriformes*

Fungal Isolate	Frequency of Occurrence (n-**98**)	Percentage Frequency of Occurrence (%)
Aspergillus sp	42	42.86%
Penicillium sp	20	20.41%
Mucor sp	25	25.51%
Rhodotorula sp	11	11.22%
Total	**98**	**100%**

Table 4.6: Cultural and microscopic examination of fungal genea

Somatic structure	Hypae	Stipes colour	Surface	Vesicle serration	Shape	Isolates
Very rough irregular	Septated	Slightly brown	Smooth walled	Biseriate large size	Glubose	*Aspergillus sp*
Round and irregular	Septated	White to blue green, gray	Flat, velvety wooly in texture	Medium size	Globose to elongated sausage shape	*Penicillum sp*
Produce columella with thick roundwall	Septated in mature hypae	White to grey to brown	Cotton white fluffy	Large size	Glubose	*Mucor sp*
Produce Blasto conidia	Form rudimentary hyphae	Coral red to Salmon coloured colonies	Mucoid to pasty tough colony	Large elongated size	Ovoidal to elongated Glubose	*Rhodotorula sp*

Table 4.7: The Phytochemical Analysis of Aqueous Extract of *Costus afer* stem

Parameters	Qualitative	Quantitative
Alkaloids (mg/100g)	+	0.614
Flavonoids (mg/100g)	++	1.401
Saponins (mg /100g)	+	3.003
Tannins (mg/100g)	+	0.971
Cyanogenic glycosides (mg /100g)	-	0.764

Table 4.8: The Phytochemical Analysis of Ethanolic Extract of *Costus afer* stem

Parameters	Qualitative	Quantitative
Alkaloids (mg/100g)	-	0.320
Flavonoids (mg/100g)	+	0.611
Saponins (mg /100g)	++	5.272
Tannins (mg/100g)	++	2.380
Cyanogenic glycosides (mg /100g)	++	0.958

Keys
+ - Scanty
++ - Moderately
- - Absent

Table 4.9:Antibiogram of Ethanolic extract of *Costus afer* stem

Test Organisms	Concentration of Ethanolic extract (mg/ml)					
	1/100	**1/2**	**1/4**	**1/8**	**1/16**	**Control**
Pseudononas sp	-	-	-	-	-	18mm
Micrococcus sp	11mm	8m	-	-	-	19mm
Streptococcus sp	-	-	-	-	-	-
Staphylococcus sp	10mm	-	-	-	-	37.5m
Proteus sp	-	-	-	-	-	-

Table 4.10: Antibiogram of Aqueous Extract of *Costus afer* stem

Test Organisms	Concentration of Aqueous extract (mg/ml)					
	1/100	**1/2**	**1/4**	**1/8**	**1/16**	**Control**
Pseudononas sp	-	-	-	-	-	18mm
Micrococcus sp	20mm	18mm	-	-	26m	19mm
Streptococcus sp	13mm	8mm	-	-	-	-
Staphylococcus sp	18mm	-	-	-	-	37.5m
Proteus sp	8mm	-	-	-	-	-

IV. DISCUSSION OF RESULTS

Antibiotic resistance has become a topic of global concern. The relationship between human norms and plants is not limited to the use of plant for food, shelter and clothing alone but also includes their use for ornamentation and health care. The clinical efficacy of many existing antibiotics is being threatened by the emergence of resistant pathogens (Etukudo, 2003).

The phytochemical and antibiogram of *Costus afer* on microorganisms isolated from the skin and different part of body fresh *Silurformes* was studied. The results obtained shows that the fin of the fresh *Silurformes* had the highest bacterial count which range from 4.6 x 10^4cfu/g to 11.2x10^4cfu/g followed by the back which range from 3.6 x 10^4cfu/g to 8.1 x 10^4cfu/g, abdomen which range from 5.4 x 10^4cfu/g to 7.2 x 10^4cfu/g and the tail which range from 3.7 x 10^4cfu/g to 5.2 x 10^4cfu/g. In fungal counts, the abdomen range from 0.5 x cfu/g to 1.1 x 10^4 cfμ/g, the back range from 0.6 x 10^3 cfu/g to 1.3 x 10^4 cfu/g, the tail had from 0.4 x 10^3 cfu/g,to 1.7 x 10^4 cfu/g, and the fins ranged from 0.2 x 10^3 cfu/g, to 1.2 x 10^4 cfu/g.

This finding agrees with the work of Ashie *et al.*, (2001) and Gram and Huss (2000). who reported high microbial diversity on fish. *Silurformes* are a diverse group of ray-finned fish they are named for their prominent barbells, slender whisker- like tactile organs near the mouth which give the image cat- like whiskers.

The bacteria genera identified from this research study are *Pseudomonas sp, Micrococcus sp, Streptococcus sp, Staphylococcus sp*. and *Proteus sp.* While the fungal isolates identified are *Aspergillus sp, Penicillium sp, Mucor sp* and *Rhodotorula sp.* This finding is in line with the works of other researchers like Gram and Huss (2000) and Gram and Dalgaard (2002) who reported the isolation, of some of the aforementioned bacterial genera isolated from fish and fish products.

Fish spoilage is as a change in fish or fish products that renders them less acceptable, unacceptable or unsafe for human consumption. Microbial growth and metabolism is a major cause of fish spoilage (Gram and Dalgaard, 2002).

Microbes such as mentioned above are found on the outer body covering and the inner surface of fresh fish such as the skin, gills, tails and gastrointestinal tract. The poikilothermic nature of fresh fish allows a wide variety of bacteria and fungi to grow including the Gram negative, rod-shaped bacteria to the genera Pseudomonas, Flavobacterium, etc and Gram positive bacteria as *Bacillus, Clostridiums, Listeria monocytogenes* (Gram and Huss, 2000). According to Olafsdottir *et al.*, (2006), who stated that *Pseudomonas sp* appeared responsible for sweet, fruity spoilage odours of fish. The isolation of *Staphylococcus sp* and *Streptococcus sp* may be attributed to handling, transportation and other environmental factors.

Costus afer is commonly used as a medicinal plant throughout tropical Africa. The stem, seeds and rhizome of *Costus afer* contains several steroidal sapogenins of which diosgenin is the most important one and this has resulted in the use of this plant as a sterilization agent during the washing and preparation of fresh *Siluriformes* before cooking and

consumption (Aguiyi *et al.,* 2004). The phytochemical analysis of *Costus afer* was also studied, and the results obtained revealed that aqueous extracts of *Costus afer* stem has more phytochemical than the ethanolic extracts as presented. The aqueous extracts quantitative parameters shows alkaloids with 0.614, flavonoids 1.401, saponins 3.003, tannins 0.971 and cyanogenic glycosides 0.764. Qualitative analysis revealed scanty concentration (+) in alkaloids, saponins, tannins, and moderate concentration of flavonoids while cyanogenic glycoside was absent. The results of the qualitative analysis of phytochemical composition of the ethanolic extract of *Costus afer* revealed moderate concentration (++) in saponnins, tannins and cyanogenic glycosides and scanty concentration (+) in only flavonoids, while alkaloids was not detected (absent). The quantitative analysis recorded the highest concentration in saponins (5.272), followed by tannins (2.380) and cyanogenic glycosides (0.958), flavonoids had (0.611) while alkanoids recorded the least concentration (0.320). According to Aguiyi *et al.,* (2004) who reported that aqueous extracts of the leaves and stem of *Costus afer* showed significant antibacterial and anoebicidal activity in vitro. A similar study by Iwu (2006) confirmed that aqueous extracts of *Costus afer* posses anti-oxidative properties as well as bioactive metabolites (Iwu, 2006).

This research on the antibiogram of *Costus afer* on microorganism isolated from the skin and other body parts of fresh Siluriformes shows that the bacterial isolates were highly resistant in all the concentrations of ethanolic extracts, especially those found in the Gram negative bacteria. This finding agrees with the work of Akpan *et al.,* (2012), who reported the resistant of gram negative bacteria against the ethanolic extracts of *Costus afer*. In aqueous extracts *Pseudomonas sp* was highly resistant in 1/100, ½, ¼, 1/8 and 1/16 concentrations. *Staphylococcus sp* was highly sensitive in 1/100 concentration while 1/8 and 1/16 were resistant. *Proteus* sp was highly resistant in all the prepared concentrations, *Micrococus sp* was sensitive in 1/100, ½ and 1/16 concentration, *Streptococcus sp* was highly resistances at all concentration. This result corroborate with the work of Akpan *et al.,* (2012), who reported that Gram positive organism was more sensitive to the plant extract of *Costus afer* than the Gram negative organisms.

The antibiotic resistance of some bacterial strains is as a result of natural resistance in certain types of bacteria genetic mutation or by species acquiring resistance from one another. According to Prescott *et al.,* (2005), resistance bacteria may either use an alternate pathway to bypass the sequence inhibited by the agent or increase the target metabolite. Some pathogens have plasma membrane translocase that expel drugs they are relatively non-specific and can pump many different drugs out of their system thereby not having any effect on them.

V. CONCLUSION

Conclusively, medicinal plants play a great role in human life and have substances that are used for traditional therapeutic and modern drug production purposes in primary health care delivery. *Costus afer* as a medicinal plant is commonly used for traditional therapeutic and other socio-cultural purposes such as wrapping indigenous food items, mat making, feed to small ruminants, treating cough, measles, malaria, eye defect, etc. From the result, *Costus afer* can be used for sterilization of fresh *Silurformes* during preparation for cooking as it is capable of reducing the level of some Gram positive organisms and enhancing the safety of the fish delicacy for consumption.

VI. RECOMMENDATIONS

- It is hereby recommended that *costus afer* can and should be extracted for use as sterilizer during the preparation of fresh fish for consumption.
- The fish should be soaked in the extract for a considerable time period before cooking
- The study recommends that fish and sea foods should be properly cooked before consumption to avoid ingestion of pathogens which might cause food borne diseases.
- There should be public education program on proper personal hygiene and the risk of consuming contaminated fish.

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