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Microorganisms in association with biofilm formation and anti biofilm capacity of fermented corn water

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Abstract
Fermented corn water aliquot was evaluated for its antimicrobial activities on biofilm organisms within Enugu Metropolis in Nigeria. Congo red agar (CRA) method was used to determine the qualitative assay and detect biofilm producing microorganisms. The identified isolates were sub-cultured on sterile nutrient agar and blood agar plates and incubated for 24 hours at 37℃. Both quantitative, qualitative phytochemicals and proximate analysis were carried out on the raw corn grains and fermented corn water respectively. The phytochemicals quantitative analysis showed that the presence of alkaloid (white corn 2.67mg/100g, yellow corn 2.06mg/100g), glycoside (white corn 2.11mg/100g, yellow corn 2.78mg/100g), Phenol (white corn 0.07mg/100g, yellow corn 0.05mg/100g), tannin (white corn 0.2mg/100g, yellow corn 1.4mg/100g), flavonoid (white corn 0.03mg/100g, yellow corn 0.56mg/100g), steroid (white corn 2.98mg/100g, yellow corn 2.55mg/100g) and saponin (white corn 3.23mg/100g, yellow corn 1.07mg/100g). The quantitative proximate analysis showed 0.98mg/100g of ash, 8.90mg/100g of moisture, 14.12mg/100g of fat, 1.15mg/100g of fiber, 10.98mg/100g of protein, 63.87mg/100g of carbohydrate. The biofilm organisms isolated were Staphylococcus *aureus, Proteus species*, *Escherichia coli*, *Bacillus species*, *Candida species* and Cryptococcus *neoformans*. The organisms isolated from fermented corn water were *klebsiella species, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus species Aspergillus fumigatus, Aspergillus muco* and *Candida species.* The biofilm isolates were tested for sensitivity to different 2mls aliquots of fermented corn water from different days of corn fermentation ranging from 3rd day to 7th day using agar well diffusion technique. The outcome showed that the longer the fermentation days the stronger the inhibitory activity on biofilm organisms. A comparative minimum inhibitory concentration between fermented corn water and antibiotic Levofloxacin 500g diluted in 100ml of sterile water was used as control. The fermented corn water showed 20mm zone of inhibition while levofloxacin indicated 35mm zone of inhibition. The results indicated that tannin showed inhibitory activity against *Staphylococcus aureus* ranging from 4mm to 20mm, *Proteus* 6mm to 28mm, *Escherichia coli* 6mm to 21 mm respectively but no activity against *Bacillus* specie even when it was reconstituted at the concentration of 0.78%, 1.56%, 3.13% 6.25%, 12.25%, 25%, 50% and 100%. The molecular analysis showed that fungi isolated from white corn has 97.62% pairwise similarity with *Penicillium citrinum* isolate ObaCOsho_13 which has NCBI accession number MN861067.1 while that of yellow corn has 99.4% pairwise similarity with *Aspergillus aculeatus* strain WZ-112 which has NCBI accession number MN856264.1 and bacteria has 90.77% pairwise similarity with *Bacillus subtilis* strain W13 which has NCBI accession number M98677.1. However, the bacteria isolated from algae biofilms has 97.03% pairwise similarity with *Escherichia coli* strain E2 which has NCBI accession number KY780337.1 and 96.72% pairwise similarity with *Enterobacter cloacae* strain EC1 which has NCBI accession number KJ210326.1. From the outcomes of this study fermented corn water has inhibitory effect on some biofilm organisms and could offer a brighter opportunity as alternative antimicrobial agents in the wake of ever evolving and rapid microbial resistance to synthetic therapeutic antimicrobial agents.

Keywords: Biofilms, anti-biofilms, phytochemicals, algae, fermented corn water, *Bacilli*, *Staphylococcus aureus*

Introduction

A biofilm is the gathering of surface connected microbial cells that are enclosed in an extracellular polymeric substance matrix [1]. Anton Van Leeuwenhoek first dictated microbes on tooth surface using ordinary microscope. However, Heukelekian and Heller observed the "bottle effect" for marine microbes, that the growth of bacteria and its actions were greatly improved by the integration of a surface to which these microbes were attached [2]. Furthermore, Zobell, C.E in 1943 noted that the quantity of bacteria on surfaces was unusually advanced than in the proximate medium [3]. Biofilm establishment and maturation is an uninterrupted, dynamic, and multifaceted procedure based on the matrix, culture medium, different cell characteristics, molecules, cell metabolism, and genetic makeup [4]. After bacteria are established either in a living or nonliving surface, it uses extracellular signaling system to communicate one another through a mechanism known as quorum sensing (QS). QS regulates the entire formation phases of biofilm, by activating regulatory genes in bacteria to produce extracellular matrices like EPS and proteins and subsequently produce comprehensive and mature biofilm structure [4]. The QS systems control the behavioral patterns of bacteria in a niche by means of signalling facilitated by auto-inducer molecules [5].

Bacteria that are pathogenic and can cause food spoilage and have the ability to form biofilm are major problem to both healthcare and food processing industries [6]. Biofilm are made up of microbial cells such as bacteria or fungi entranced in an extracellular matrix that consists of polysaccharides. Extracellular DNA and other elements which is self-produced. When compared to free living cells in suspension known as planktonic cells, cells in a microbial biofilm are much less vulnerable to antimicrobial agents and this lowers sensitivity which creates negative effect on the therapeutic management of biofilm-associated diseases [7]. It

is projected that about 65% to 80% of all chronic diseases are biofilm associated. Apart from the usual antimicrobial resistance mechanisms of biofilms, various tolerance mechanisms of microbial biofilms are linked to susceptibility reduction, decrease in diffusion of antimicrobial agent into biofilm, altered chemical microenvironment within the biofilm, resulting in areas of no growth or slow growth, adaptation to stress response mechanisms, existence of community of small but with high resistant lingering cells, and adaptations to metabolic pathways [8].

Properties of Biofilm cell shows that cell surface hydrophobicity, existence of flagella and fimbriae, and the making of EPS affects the proportion and degree of attachment of microbial cells. Fimbriae contributes to cell surface hydrophobicity and attachment, possibly by overpowering the initial electrostatic repulsion barrier that exists between the cell and substratum [9]. Furthermore, biofilms are extremely heterogeneous, comprising of micro colonies of bacterial cells encased in an EPS matrix and separated from other micro colonies by interstitial voids (water channels) [10].

The expression of genes in biofilms is practically controlled by the dynamic of physicochemical factors external to the cell and may involve complex regulatory pathways [11]. Biofilms as well offers a model microbial niche for the inter change of extrachromosomal DNA -plasmids [12]. Plasmid-carrying strains as well have been identified to transfer plasmids to recipient microorganisms, and the outcome forms biofilm; however, in the absence of plasmids these same microorganisms form only micro colonies and stagnates its formation. Meanwhile plasmids could encode for resistance to numerous antimicrobial agents, biofilm connections as well offers a process for choosing and enhancing the spread of, bacterial resistance to antimicrobial agents [13]. It's estimated that more that 90% of bacteria species that are infectious such as *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) have natural capability to form biofilm, thus presenting biofilms as the major reason for multiple drug resistance [14] Occasionally, bacteria within biofilms are subjected to predation by other organisms such as free-living protozoa, bacteriophage, and polymorphonuclear leukocytes (PMNs) due to concentration of restricted cell [15].

Algae are photosynthetic microorganisms that has the ability to transform the anaerobic environment into an aerobic by the procedure known as oxygenic photo-phosphorylation [16]. Algae are also eukaryotic, photosynthetic, unicellular, or multicellular microorganisms. They mostly grow in moist environment and not true plants and found in the soil, ponds, rivers and lakes [17]. Algal biofilm is a typical model system for studying the synergy among different prokaryotic-eukaryotic organisms and their associations in the soil with emphases to their potential to cause diseases [16]. In carrying out photosynthesis algal biofilm produce oxygen and consume dissolved carbon dioxide [18]. Biofilm produced by microalgae are extracellular polymeric substances made up of polysaccharides, proteins, nucleic acids and lipids. These EPSs stops the cells and makes the biofilm to stabilize, facilitating bonds to the solid surfaces [19].

Anti biofilm is either a natural or stimulated procedure that could lead to decrease of microorganisms in biomass by altering biofilm establishment structure and features [20]. There are two different mechanisms used by anti-biofilms to regulate the establishment of biofilms. First by inhibiting bacteria attaching to the surface and secondly, through irreversible damage of bacteria attaching to the surface. Anti-biofilm agents are categorized into synthetic and natural [20]. Fungal biofilms such as *C. albicans* are basically made up of yeast form and hyphal cells, these are needed for the formation of biofilm. However, biofilm formation is a successive procedure that led to adherence to a substrate such as abiotic or mucosal surface, multiplication of yeast cells on the solid surface as well as initiation of hyphal formation [21].

Phytochemicals are broadly five classes of natural compounds that have high anti-biofilm properties. These are phenolics, essential oils, terpenoids, lectins, alkaloids, polypeptides, and polyacetylenes. Phenolics are a group of compounds and are made up of seven subclasses which include phenolic acids, quinones, flavonoids, flavones, flavonols, tannins, and coumarins, out of which tannins, specifically condensed tannins, have anti biofilm activity [22]. Phytochemicals have the capability to inhibit the progression and access to essential nutrients required for adhesion as well as the growth of bacteria [22]. Hence the objective of the study to determine the antimicrobial activities of fermented corn water on biofilm and biofilm organisms.

Experimental Section

Materials and methods

Sample Collection

Corn samples were purchased from several commercial points (Abakpa Market, Ogbete Main Market, New Market, and Afia 9 Market) within Enugu metropolis. The samples of both yellow and white maize grains purchased were transported in a sterile container to Enugu State University of Science and Technology Microbiology laboratory for analysis. Algae samples were collected from a moistured sandy soil in a sterile container from these locations: Abakpa, New Heaven, Ogbete, New market, Uwani and Independence Layout. Both bacteria and fungi were isolated from the algae samples and fermented corn water.

Glass Wares

All the glassware used were sterilized in a hot air oven maintained at 100°C for one hour, while other materials used were sterilized in an autoclave maintained at 121°C for 15 minutes.

Media Preparation

Nutrient Agar About 7g of nutrient agar powder was weighted out and dispensed in 250ml of distilled water in a conical flask. Allowed to dissolve completely in distilled water for at least 10minutes with continuous agitation, thereafter, sterilized using autoclave at 15lb/sqm at 121°C for 15minutes. The sterilized media was allowed to cool to 45-50°C and then dispensed into sterile petri dishes 20ml each. The plates were left to solidify and kept until required. The nutrient agar was prepared according to the manufacturers guide.

Blood Agar

Forty (40) grams of the prepared medium was added to 1000 ml distilled or deionized water. The suspension was heated up to boiling to dissolve the medium completely. It

was then sterilized by autoclaving it at 15 lbs pressure and 121°C for about 15 minutes. The medium was then taken out of the autoclaved and cooled to about 40-45°C. To this, 5% v/v sterile defibrinated blood was added aseptically and mixed well. The media was then poured into sterile Petri plates under sterile conditions. Once the media solidified, the plates were placed in the hot air oven at a lower heat setting for a few minutes to remove any moisture present on the plates before use. This was prepared according to the manufacturers guide.

Congo Red Agar (CRA) Method

Congo Red Agar (CRA) method was used as described by Freeman *et al*., [23]. The CRA medium was constructed by mixing 0.8 g of Congo red and 36 g of sucrose to 37 g/L of Brain heart infusion (BHI) agar. After incubation period of 24 hours at 37°C. This was prepared according to the manufacturers guide [24].

Peptone Water

About 1.9g of peptone was weighted out into sterile conical flask. Then dissolved in 125ml of distilled water, it was properly agitated to have a uniform mixture. Then 10ml each was aseptically dispensed into test tubes. Each test tube was properly sealed with cotton wool and foil paper and arranged into a beaker and sterilized using autoclave at a temperature of 121°C for 15 minutes. The sterilized media was allowed to cool and then stored in the refrigerator until required. Prepared according to the Manufacturers guide.

Preparation of McFarland Solution with Standardization of Inoculums

Following manufacturer's guide.0.5 McFarland solution was prepared by mixing 0.05g of 1.75% barium chloride dehydrate (BaCl2.2H2O) with 9.95ml of 1% sulphuric acid (tetraoxosulphate VI acid, H2SO4) in a test tube. 10ml of normal saline were placed on freshly sterilize test tube and it was incubated with the testing bacterial each on a single tube containing 10ml of saline and mash it with McFarland solution.

Phytochemicals and Proximate Analysis

The corn samples were washed with distilled water. It was dried at room temperature and grounded into powdering form using electric blender. The proximate analysis of the crude extract for moisture content and ash content was performed using the AOAC [25]. This was done by Fedrah Research Institute, Emene Enugu State Nigeria.

The nitrogen content was calculated using the micro-Kjeldahl method, and the nitrogen content was multiplied by a factor of 6.60 to convert it to protein. After subtracting the total percent of other food leavesrients from 100 percent, the total carbohydrate content was calculated. The moisture content was determined as well as ash content, crude protein, and carbohydrate content.

Qualitative Methods of Phytochemicals

Alkaloid was tested using Wagner reagent test (iodine-potassium-iodide solution) gave reddish Brown colour precipitate.

Test for Glycosides was carried out using Borntrager's Test and turned from Pink to red color and shows the presences of glycosides.

The presence of phenol was determined using Million reagent, the color changed to red indicating the presence of phenol.

Test for Saponins (FROTHING TEST), the presence of saponin was determined using fronthing test the characteristic honeycomb like froth indicated saponins presence.

Test for Tannins Shinoda test a greenish coloration indicated the presence of Tannin.

Test for Quinones, black color formation indicates the presence of quinoid compound or iodine colour (blue black) or a brick red precipitate.

Test for Flavonoid Lead acetate formed yellow color precipitate.

Salkowaski test was used to test for Steroids and the developed red color in chloroform layer indicating the presence of steroids.

Liebermann–burchard test indicated that blue colour exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of steroid.

The Temperature was determined using thermometer. The thermometer was dipped into the sample solution until the values remains constant before reading were taken.

The total titratable acidity (TTA) was determined by taking ten millilitre aliquots of the samples using pipette and titrated against 0.1 M NaOH solution to phenolphthalein end point and the acidity was calculated as g lactic acid / 100%.

The pH was determined by taking ten grams of sample mixed in 100 ml of CO2 free distilled water. The mixture was allowed to stand for 15 min then shaken at 5 minutes interval and filtered with Whatman No. 14 filter paper. The pH of the filtrate was measured in duplicate using a pH meter (Model HM-305, Tokyo, Japan) AOAC [26].

The Oxalate was determined by using the method of AOAC [27].

Phytic acid was determined by the procedure AOAC [27].

Cyanogenic glycoside was determined using alkaline picrate method of Onwuka and Olopade [28].

Corn Fermention Process

A total of 1000grams of each variety of maize grains was weighted from each sampling site. The physical properties of the corn samples were determined by measuring the temperature, corn pH, moisture content, colour determination and textures. The grains were washed using sterile distilled water. The corn grains were put in a sterile container and steeped in a clean water for a period ranging from 1st to 7th day respectively. After the initial steeping for 3days (72hours) the sample of the corn water (aliquot) 2mls was collected and decanted and repeated for 4th day, 5th day, 6th day and 7th day respectively [29]. The steeping was done using traditional method by allowing the corn grains to ferment in the water for the required number of days [30].

Microbial Analysis

This was carried out using a ten-fold serial dilution to enumerate the microorganisms present by the spread plate method. Nutrient agar and Blood agar were used for bacteria growth. After incubation, the plates were examined for colonies using their cultural characteristics.

The fungal count was recorded using a colony counter. The different colonial morphologies obtained were recorded by visual observation. Pour culture of identified isolates were made. These colonies were sub-cultured onto fresh media

usung streaking method with an inoculating loop or wire loop to obtain pure cultures [29]. After sub culturing, a small portion of each of the sub-cultured colony was cut using a sterile scalpel and placed on a sterile glass slide using a Sterile Forceps Then, covered with a cover slip and placed in a petri dish containing moistened cotton wool swabs. This was allowed to stand for 3days at 25^{O}C. afterwards, the cover slips were removed with a forcep and placed on glass slides containing lactophenol-cotton blue stain. This was observed under the microscope to identify the shape, structure of conidia, pigmentation [30].

Biochemical Analysis

Biochemical tests were carried out using Gram staining, catalase test, coagulase test, indole, methy red. citrate test and oxidase test This was done according to Nwadioha *et al*., [31].

Anti Microbial Test

The sterilized filter discs papers measured 6mm were saturated with fermenred corn water and was allowed to dry for 24 hours respectively. The discs were then harvested and dried in a hot air oven for 30℃ for 1hour.

The solutions of the isolates were prepared by using the Mcfarland standard concentrations. The organisms were incoluated into sterile media plates respectively. And were spread out using sterile bemt glass rods there after the discs were seeded in the plates and incubated at the apporopirate temperature.

The antimicrobial assay of fermented corn water was carried out using three methods which were pour plate, agar well and disc diffusion methods.

Using the micropipettes a total of 0.1ml of broth culture of the test organisms (*Staphilococus aureas*, *Echericia coli*, *Protus*, and *Bacilli)* was incubated in 9.9ml of various corn water aliquoted from different days of corn steeping. Thereafter, 0.1ml of the preparation of the respective extracts with test organisms were inoculated into different sterile petri dishes and overlaid with sterile molten nutrients agar and mixed by rotating in clockwise and anti clock wise direction. The incubated for 24-48 hours at 37℃.

Serially diluted broth culture 10^4,10^6,10^8,10^{10} of test organisms were inoculated into sterile nutrient agar and spread using a sterile swab stick. Sterile cork borer was used to make five wells in each plate. Therefore, 0.1ml of each extract were carefully pipette into the wells. The preparations were incubated for 24hours at 37℃, then the preparation was observed for zones of inhibition and zones were measured and recorded.

Serially diluted broth culture 10^4,10^6,10^8,10^{10} of test organisms were inoculated into already prepared nutrient agar plates respectively, spread out using sterile swab stick. The already prepared sensitivity discs were placed at different location on the plates and incubated for 24-48hours at 37℃, then zones of inhibition were observed, measured and recorded.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration was determined using agar well technique. Serial dilution of the various extracts which showed zones of inhibition on the test organisms was made with their respective solvents. Total of 0.1ml of dilatated extracted were incorporated at varying concentration into the agar plate containing the test organisms (10^4,10^6,10^8,10^{10}). The plates were incubated at 37℃ for 24hours. The lower concentration of extracts that had zones of inhibition within the incubation period was taken to be the minimum inhibitory concentration. The diameter of zones of inhibition were measured and recorded accordingly.

Antimicrobial Activities of Fermented Corn Water on the Algae Biofilm

Treatment of the algae with fermented corn water

Antimicrobial Disc Preparation using fermented corn water. A perforator was used to perforate about 6mm of Whatman No.1 filter paper, the discs were wrapped in foil paper and placed in a beaker covered with foil then sterilized using hot air oven at 80℃ for 1 hour and the sterile discs were stored in the refrigerator until when needed.

Molecular Test

Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio-Edit software and MEGA X were used for all genetic analysis was done by bioinformatics Ibadan Nigeria.

Results

Table 1: Phytochemical Qualitative Results of Raw White and Yellow Corns

Results of phytochemical qualitative analysis		
Parameters	**White Corn**	**Yellow Corn**
Alkaloid	+ + +	+ + +
Glycoside	+ + +	+ + +
Phenol	NIL	NIL
Tannin	+	+ +
Flavonoid	NIL	+
Steroid	+ + +	+ + +
Saponin	+ + +	+ +

+ indicates low + + indicates moderate + + + indicates high

Phytochemical Qualitative Results of both raw white and yellow corns indicated high levels of Alkaloid. Glycoside, Steroid and Saponin, white corn showed low level of tannin while yellow corn showed moderate level of tannin. Both white and yellow corns showed zero level of Phenol.

Table 2: Phytochemical Quantitative Results of Raw White and Yellow Corns

Results of phytochemical quantitative analysis(mg/100g)		
Parameters	**White Corn**	**Yellow Corn**
Alkaloid	2.67	2.06
Glycoside	2.11	2.78
Phenol	0.07	0.05
Tannin	0.2	1.4
Flavonoid	0.03	0.56
Steroid	2.98	2.55
Saponin	3.23	1.07

Phytochemical Quantitative Results showed that Saponin was highest in white corn with 3.23 mg/100g. Other high Phytochemical Quantities were Akaloid, Glycoside and steroids. Others were Phenol, tannin and favoid that ranged from 0.2 to 1,4 mg/100g in both white and yellow corns.

Table 3: Results of proximate quantitative analysis (mg/100g)

Parameters	Yellow corn
Ash	0.98
Moisture	8.90
Fat	14.12
Fiber	1.15
Protein	10.98
Carbohydrates	63.87

Results of proximate quantitative analysis showed that Carbohydrates content was highest with 63.87mg/100g while the ash content is lowest with 0.98mg/100g.

Table 4: Microoganisms Isolated from sub culture of Algae Biofilm

S. No.	Microorganisms	Morphology
1	Staphylococcus aureus	Golden yellow colonies
2	Escherichia coli	Smooth, circular, grayish white colonies
3	Proteus species	Rod shaped
4	Bacillus species	Rough circular opaque colonies
5	Candida species	Small creamy white colonies
6	Cryptococcus neoformans	Whitish mucoid colonies

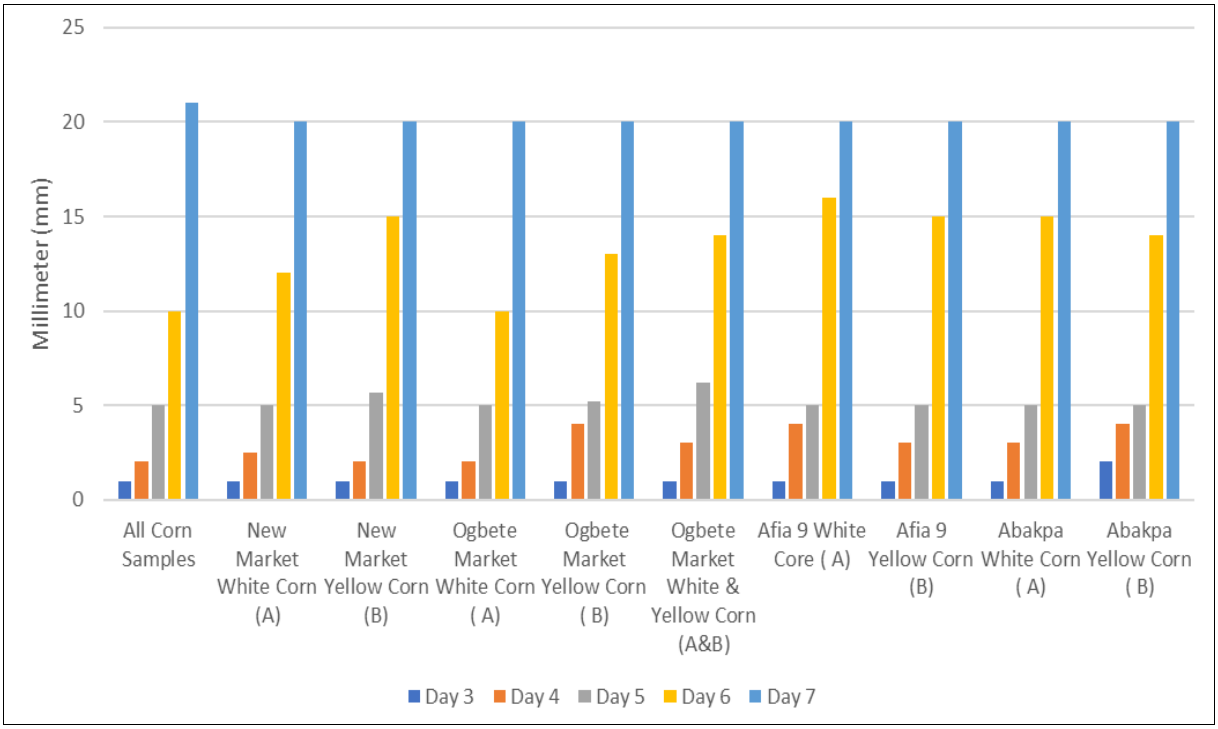

Fig 1: Graph showing the Sensitivity of Fermented Corn Water to Algae Biofilm Isolates Per Day

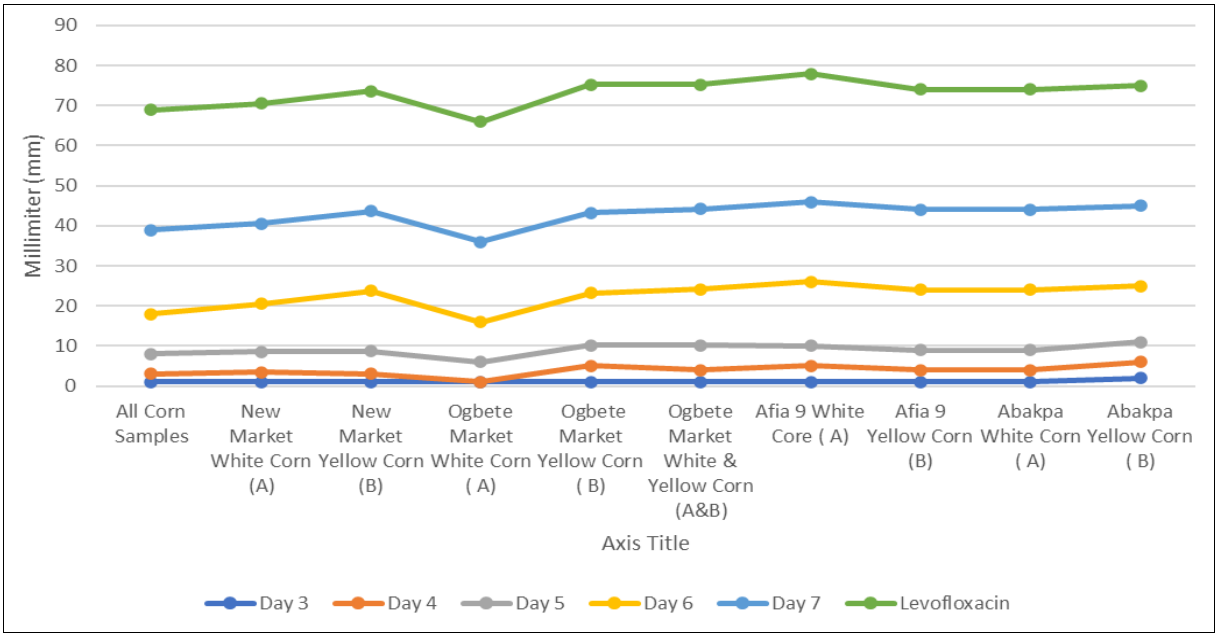

Fig 2: Graph showing the Comparison of the Sensitivity of Fermented Corn Water and Levofloxacin to Biofilm

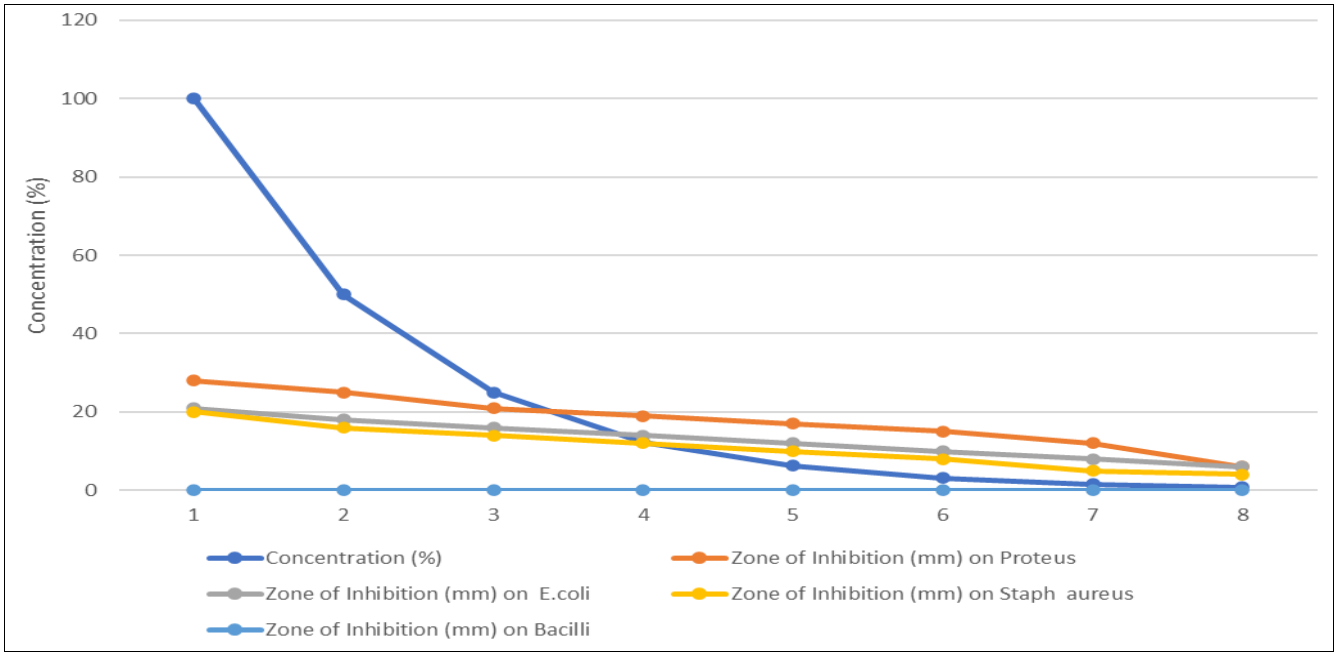

Fig 3: Graph Showing the Sensitivity of Fermented Corn Water to Biofilm Organisms

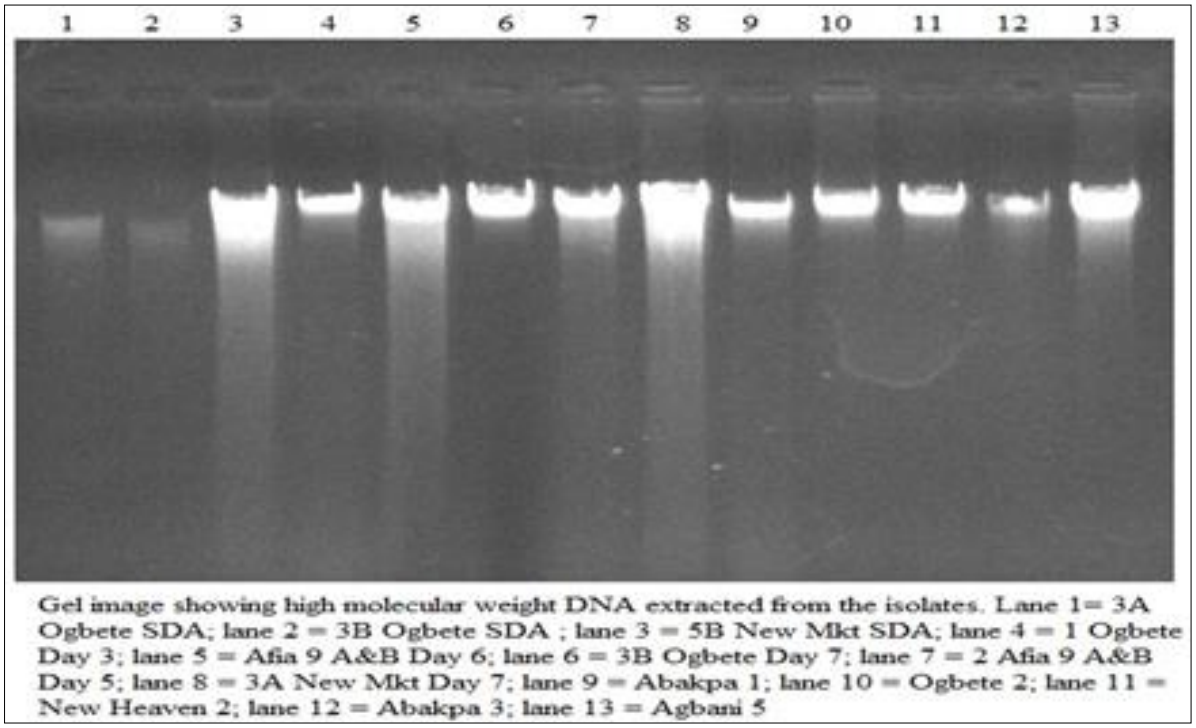

Fig 4: Gel Image showing high molecular weight DNA extracted from the Isolate

Discussion

Phytochemical qualitative results of both white and yellow corns indicated high levels of alkaloid, glycoside, steroid and saponin. While tannin was low in white corn and moderate in yellow corn. There was no flavonoid in white corn but low in yellow corn. However, there was no phenol in both white and yellow corn (Table 1).

The phytochemical quantitative analysis showed the presence of alkaloid (white corn 2.67mg/100g, yellow corn 2.06mg/100g), glycoside (white corn 2.11mg/100g, yellow corn 2.78mg/100g), Phenol (white corn 0.07mg/100g, yellow corn 0.05mg/100g), tannin (white corn 0.2mg/100g, yellow corn 1.4mg/100g), flavonoid (white corn 0.03mg/100g, yellow corn 0.56mg/100g), steroid (white corn 2.98mg/100g, yellow corn 2.55mg/100g) and saponin (white corn 3.23mg/100g, yellow corn 1.07mg/100g) (Table 2).The quantitative proximate analysis showed 0.98mg/100g of ash, 8.90mg/100g of moisture, 14.12mg/100g of fat, 1.15mg/100g of fiber, 10.98mg/100g of protein, 63.87mg/100g of carbohydrate. (Table 3).

The Figure shows the zones of inhibition of fermented corn water per day from both white corn and yellow corn. The Fermented corn water aliquot of the third day showed zone of inhibition that ranged from 1mm to 2mm, the fourth day showed zone of inhibition of 2mm to 4mm, the fifth day showed zone of inhibition of 5mm to 6.2mm, the fermented corn water of the six day showed zone of inhibition of 10mm to 16mm while the seventh day showed 20mm (Fig 1).

The Fig showed the comparison zones of inhibition of fermented corn water per day from both white corn and yellow corn and 500mg Levofloxacin antibiotic. The Fermented corn water aliquot of the third day showed zone of inhibition that ranged from 1mm to 2mm, the fourth day showed zone of inhibition of 2mm to 4mm, the fifth day showed zone of inhibition of 5mm to 6.2mm, the fermented corn water of the six-day showed zone of inhibition of 10mm to 16mm while the seventh day showed 20mm. The 500mg of Levofloxacin showed zone of inhibition of 30mm to 32mm (Fig 2).

The zone of inhibitions of fermented corn water to biofilm organisms showed that *Staphylococcus aureus* was 8mm on the 3rd day, 10mm from 4th to 6th day and 20mm on the 7th day. The fermented corn water zone of inhibition on *E. coli* showed 5mm on the 3rd day, 8mm on the 4th day, 10mm on the 5th day, 15mm on the 6th day and, 20mm on the 7th day. The fermented corn water zone of inhibition on *Pseudomonas* showed 3mm on the 3rd day, 5mm on the 4th day, 5mm on the 5th day, 5.8mm on the 6th day and, 10mm on the 7th day. *Proteus* was 10mm from 3rd to 5th day, 15mm on the 6th day and 25mm on the 7th day. The fermented corn water had not zone of inhibition on *bacilli*. It's most sensitivity to *proteu*s, followed by *Staphylococcus aureus* and *E. coli*. These organisms were identified by biochemical test, gram stain and cultural characteristics (Fig 3). The increasing antimicrobial resistance of pathogens to anti biotics necessitated the development of antimicrobial agents from other sources [32] and that is the main objective of this research work. The gel showed high molecular DNA extract from the isolates in the different markets and lanes and (Fig 4). The Minimum Inhibition Concentration (MIC) of fermented corn water showed inhibition to biofilm organisms. The outcome of this study is comparable to the results obtained by Rasha *et al.,* [33] which showed that extracts from Aquatic Zea Mays have a very high inhibition effect against gram positive bacteria such as *Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis, Staphylococcal pneumonia, Staphylococcus agalactiae, Staphylococcus mutants* and *Enterococcus feacalis* with inhibition zones that ranged from 26mm and 32 mm.

The molecular analysis showed that fungi isolated from white corn has 97.62% pairwise similarity with *Penicillium citrinum* isolate ObaCOsho_13 which has NCBI accession number MN861067.1 while that of yellow corn has 99.4% pairwise similarity with *Aspergillus aculeatus* strain WZ-112 which has NCBI accession number MN856264.1 and bacteria has 90.77% pairwise similarity with *Bacillus subtilis* strain W13 which has NCBI accession number M98677.1. However, the bacteria isolated from algae biofilms has 97.03% pairwise similarity with *Escherichia coli* strain E2 which has NCBI accession number KY780337.1 and 96.72% pairwise similarity with *Enterobacter cloacae* strain EC1 which has NCBI accession number KJ210326.1

Conclusion

This study demonstrated that the antimicrobial activity of fermented corn water on some biofilm organisms such as *Staphylococcus aureus, Proteus* and *Escherichia coli*. The results also indicated that the fermented corn water have no antibacterial effect on the *bacilli*. It proved that fermented corn water is an effective natural anti-microbial agent and could be alternative to drug resistant synthetic therapy. Though, different organisms in the same community form biofilm as a mechanism for survival or defense which becomes a major danger to health care system due to drug resistance. Therefore, fermented corn water provides phytochemical compounds with sufficient antibiofilm capacity and this natural source of antibiofilm can alleviate infections caused by biofilms. Also having understanding of the potential of natural product such as fermented corn water can lead to the discovery of antimicrobial drug.

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