

Effect of Plant Extract on Pathogens Isolated from Water Source

Abstract

This study was designed to evaluate the effect of *Aloe barbadensis Miller* (Aloe vera) and *Cymbopogon citratus* (Lemongrass) extracts on pathogenic bacteria isolated from surface and ground water samples in Port Harcourt Metropolis. Samples of Aloe vera and Lemon grass were collected, pre-treated and extracted using n-hexane and ethanol respectively. The plant extracts were qualitatively and quantitatively assessed for their phytochemical properties using standard methods. Surface and ground water samples were collected and characterized for their physico-chemical and microbiological properties using standard methods. The pathogenic bacteria isolated from the water samples were subjected to antibacterial susceptibility tests using selected commercial antibiotics and the plant extracts respectively. Result revealed the presence of eight phytochemicals namely alkaloid, flavonoid, saponnin, tannin, glycoside, steroid, phenol and terpene in both hexane and ethanol extracts of the Lemongrass and Aloe vera with varying degrees. Four bacterial pathogens namely *Streptococcus* spp., *Shigella* spp., *Pseudomonas* spp. and *Staphylococcus* spp. were identified in the water samples. All of them were susceptible to both hexane and ethanol extracts of the Lemongrass and Aloe vera plants with zones of inhibition ranging from 10.2 mm to 14.5 mm and 16.7 mm to 20.9 mm and 4.2 mm to 9.5 mm and 6.7 mm to 11.2 mm respectively. Commercial antibiotics such as Ciproflox, Streptomycin, Chloramphenicol and Cuntamycin were active against all the bacteria pathogens, with zones of inhibition ranging from 15.7 mm to 21.2 mm, 13.2 mm to 21.3 mm, 3.1 mm to 14.5 mm and 3.3 mm to 14.7 mm respectively. The antibacterial action of ethanol extract of the Lemongrass on all four pathogens was comparable to that of commercial antibiotics such as Ciproflox, Streptomycin and Ofloxacin which also demonstrated higher antibacterial action on the pathogens. This study has revealed the usefulness of Aloe vera and Lemongrass plants in phyto-antibiotics and phyto-medicine.

Keywords: Aloevera, lemongrass, surface and ground water, phyto-antibiotic

Introduction

Worldwide, infectious diseases are still a major health challenge and responsible for around 41% of the global disease burden [1]. Nigeria is characterized by speedy growth in population dynamics, inadequate access to electrical power and clean water supply together with an unfettered suburbanization. The loss of human lives due to waterborne infections in Nigeria are becoming a threat to public health safety within the country [2,3]. The liability of ailments linked to drinking contaminated water in Nigeria is massive, predominantly in rural populations where the systematized source of drinkable water is inadequate. Common waterborne illnesses in Nigeria include dysentery, diarrhea, cholera, typhoid fever and schistosomiasis among others. Diseases such as these are usually associated with bacteria, protozoans, viruses and helminthes[4,5].

Despite governmental hard work towards reducing these types of diseases, the problem seemed to be progressively persistent. The spread of developed microbial resistance to

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antibiotics is one of the major reasons for this problem. This is making not only Nigeria but the world at large to face a serious threat to the balance of public health in the form of both epidemics and pandemics of antibiotics resistance [6].

This problem has led attention to be shifted to bioactive compounds found in plant species used in herbal medicine because they have been demonstrated to possess potent antifungal, anti-viral, antibacterial, anti-helminthic and anti-protozoal properties [7,8]. The antibiotics features of these types of plants are directly correlated with their ability to produce various kinds of secondary metabolites which seem to act against bacteria, fungi, viruses, helminthes and protozoans [10].

Generally, plant extracts epitomize a constant effort to find new bioactive compounds which act against both economic and healthcare significance [9,11]. Nowadays, a significant amount of new antibiotics introduced to the global market are acquired from natural sources such as medicinal plants [12,13]. Aloe Vera is a resilient, perpetual, tropical, drought-resistant, cactus-like succulent plant which belongs to the Liliaceae family. Traditionally, the plant has been used for a wide range of therapeutic purposes [12,13]. Among the 400 species of this genus, *Aloe barbadensis* Miller was found to be the most bioactive [14]. Many of the health benefits connected to Aloe Vera have been credited to the bioactive secondary metabolites found in the gel of the leaves. These bioactive compounds which mainly include anthraquinones and polysaccharides, may act alone or in synergy with one another [15-17].

Aloe vera is a miracle plant with an abundance of health profits. This plant is likely to cure various types of skin burns, trivial cuts, and possibly cancer of the skin. Its peripheral application within the cosmetic ecosystem predominantly makes it to act as a skin healer, preventing epithelial tissue injury, a treatment for acne and provides a young-looking radiance to the skin. Aloe vera juice is anabolic and a potential detoxifier in action, taken internally, it works against various kinds of ill-health conditions such as cirrhosis, hepatitis, urinary tract infections, prostate complications, congestion, indigestion, stomach ulcers, colitis and hemorrhoids [12,17]. The pharmaceutical importance of this plant is in its gel-like pulp acquired by peeling its leaves. Commercially, Aloe can be found in drugs, sprays, ointments, lotions, liquids, drinks, jellies, soaps and creams [16].

Popularly known as citronella grass, Lemongrass is a plant that is classified under the family of Poaceae and the genus *Cymbopogon* due to the flowery organization of its grass. There are approximately 140 species classified under genus *Cymbopogon*, spread across the tropical and semi-temperate regions of Asian, African, American, Australian and European continents [18]. The members of this genus are known as aromatic grasses because they produce volatile oils with a robust lemon scent which results from the high citral content of the oils [19]. The high content of citral makes the oil a useful source for the production of beta carotene and vitamin A [20]. Lemongrass oil is primarily derived from three different species namely *Cymbopogon flexuosus*, *C. citratus*, and *C. pendulus*. The fragrance of these oils enables their use in cosmetics, perfumery and food industries [18,20]. Several studies suggest that Lemongrass owns several pharmacological properties which include pesticidal, insecticidal, herbicidal, anti-depressant, analgesic, anti-pyretic, anti-septic, astringent, fungicidal,

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bactericidal, aroma therapeutic, anti-amoebic, antidiarrheal, anti-helminthic, anti-inflammatory, antimalarial, anti-mutagenicity, antiviral, anti-cancer, anti-oxidant, hypoglycemic and neuro-behavioral properties [21-23].

Bioactive compounds found in medicinal plant species Aloe Vera and Lemongrass may provide a potential solution for combating and alleviating the problem waterborne diseases. Therefore, this study aimed to determine the effect of Aloe Vera and Lemongrass extracts on pathogenic bacteria isolated from both surface and ground water samples.

Materials and Methods

Collection and pretreatment of plant samples

Whole plant of *Aloe barbadensis* Miller (*Aloe vera*) and *Cymbopogon citratus* (Lemon grass) were collected from within Choba Campus located at the University of Port Harcourt in River State, Nigeria. After collection, the plants were cut into smaller pieces, sun-dried, powdered and extracted using n-hexane and ethanol respectively [24 [Unachukwuet *al.*, 2017; Ewansihaet *al.*, 2021)].

Preparation of plant extracts

Three hundred and fifty grams (500g) of each of the pulverized plants was weigh up using a weighing balance into four 1500ml capacity conical flask. One litre (1L) each of the solvents (n-hexane and ethanol) was added to each of the pretreated plant samples inside the 1500ml capacity conical flasks respectively. The flasks holding the blends were positioned on a shaker for 72 hours. After shacking and mixing for 72 hours, the contents of the flasks were strained using muslin cloth and then re-strained with a tripled muslin cloth. The filtrates were concentrated using a rotary evaporator and then dried on evaporating dishes at a temperature which ranged from 50°C to 60°C to viscous fluid forms. The extracts were then stored in well-corked universal bottles [24,25].

Phytochemical screening of Lemongrass and Aloe vera extracts

Phytochemical analyses of the lemongrass and Aloe vera extracts for phenols, flavonoids, tannins, saponins, cardiac glycosides, terpenoids, steroids and alkaloids were conducted using the methods described by Ezeonu and Ejikeme (2016), Khalid *et al.* (2018), Gupta *et al.* (2019) and Ewansihaet *al.* [25]

Quantitative determination of phytochemical constituents

The phytochemicals found to be present in the Lemongrass and Aloe vera extracts were quantified using the analytical methods for quantitative determination of phytochemicals found in plants in accordance with the description laid out by Ezeonu and Ejikeme [26].

Collection of the water samples

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Composite samples of surface water were aseptically collected into sterile 500mL Duran Schott glass bottles from various sampling points at River Elioizu and Iwofe waterfront by directly sinking the bottles into the surface of the water bodies. Furthermore, midstream ground water composite samples were collected directly into the same type of sterile bottles from the borehole taps located at King Jaja hostel (block A and D) in Delta Campus and Amina Kano hostel (Block A and B) in Choba Campus of the University of Port Harcourt. The samples were properly labelled and conveyed on ice to the laboratory for both physicochemical and microbiological analyses.

Determination of physicochemical parameters of the water samples

The water samples were analyzed for their physicochemical parameters by using standard methods as described by Jannat *et al.* [27] and Onyegeme-Okerenta *et al.* [28]. Parameters monitored were pH, temperature, turbidity, electrical conductivity, nitrate, phosphate and biochemical oxygen demand (BOD₅).

Isolation and characterization of bacteria in the water samples

The culture media used for the enumeration of bacteria in the water samples included Eosin Methylene Blue (EMB) Agar, Mannitol Salt Agar (MSA), MacConkey (MAC) Agar, Salmonella Shigella Agar (SSA) and Cetrimide Agar Base (CAB) respectively. Each of the culture media was aseptically prepared and plated according to the manufacturer's (HiMedia Laboratories) instructions. After the agar plates were prepared, 1mL of the respective water samples was aseptically and serially diluted with sterile distilled water up to 10⁻⁵. From each dilution, 1ml of the samples was aseptically inoculated on the agar plates in duplicates using the spread plate technique. The plates were incubated at 37°C for 24 h. After the 24 h period, the colonies which appeared on the agar plates were enumerated and recorded in colony forming unit per milliliter of the water sample (Adamu [29]).

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Following aseptic techniques, ten-fold serial dilution of the water samples up to 10⁻⁵ was conducted from which 1ml was spread onto agar plates which were prepared according to the manufacturers (HiMedia Laboratories) direction. The plates were subsequently incubated at 37°C for 24 hours. Isolated colonies were noted and purified to obtain pure culture by repeated sub-culturing on fresh agar media used for the primary isolation as described by Adamu *et al.* [29]. Pure stock cultures acquired were inoculated on slants containing the respective media and preserved in the refrigerator at 4°C until needed for further characterization and identification.

To characterize and identify the bacteria isolated from the water samples, each of the isolates were subjected to morphological assessment and biochemical analyses such as Gram staining, Catalase, Oxidase, Citrate, Glucose, Lactose, Indole, Mannitol, Methyl red, Voges Proskauer, H₂S production, Gas production, Hemolysis and Motility tests as described by Begum *et al.* [30] and Sirisha *et al.* [31]. After characterization, the bacterial isolates which demonstrated hemolytic properties were considered to be potential pathogens and further subjected to antibiotic, MIC and MBC reaction tests.

Antibacterial assay of the crude extracts

Antibacterial screening of the plant extracts was conducted as described by Unachukwuet *al.* [24] and Ewansihaet *al.* [25] with minor modifications. Zero point six grams (0.6g) of the n-hexane and ethanol extracts of the Aloe vera and Lemongrass were weighed and dissolved in 30ml each of sterile distilled water. This gave a concentration of 20mg/ml each. Mueller Hinton Agar plates were prepared according to the manufacturer's (HiMedia Laboratories) directions, streaked uniformly and labelled appropriately according to the number of suspected pathogenic bacteria isolated and identified from the water samples. Sterile cup borer (6mm) was employed to bore holes inside the culture media. The bottom of each of the wells were sealed with a drop of molten agar so as to avoid unwelcome dispersion of the plant extracts. In a drop-wise manner, 1ml of the ready-made plant extracts was added into each of the wells in droplets and the cultures were permitted to stand for 30 minutes before they were relocated to the incubator. The cultures were incubated for 24 – 48 hours at 37°C. Control plates were also arranged according to the number of the suspected pathogens isolated minus the extracts and the zones of inhibition were measured to the nearest millimeter.

Standard antibiotic susceptibility test

Disc diffusion method was used in this test as described by Ewansihaet *al.* [25]. Six millimeter (6mm) of commercially prepared antibiotic paper discs were used. The antibiotic discs used and their concentrations were Ofloxacin (10mg), Peflacin (10mg), Ciprofloxacin (10mg), Augmentin (30 mg), Cefotaxime (10mg), Streptomycin (30mg), Septrin (30mg), Ampicillin (30mg), Norfloxacin (10mg), Amoxicillin (20mg), Rifampicin (20mg), Erythromycin (30mg), Chloramphenicol (30mg), Ampiclox (20mg) and Levofloxacin (20mg) for both Gram-negative and Gram-positive bacterial isolates which were suspected to be potential pathogens. The discs were applied in accordance to the National committee for clinical laboratory standard. The antibiotics used in the test were chosen after a preliminary survey and to reflect the range of drugs commonly prescribed for the treatment of waterborne bacterial pathogens.

Sterile swab sticks were used to transfer the bacteria isolates into tubes containing physiological normal saline to form a suspension. Already prepared Mueller Hinton Agar plates were inoculated appropriately with the bacterial isolates by dipping the sterile swab sticks into the suspension and removing excess inoculum by pressing and rotating the swab firmly against the side of the tube. The inocula were streaked all over the surface of the media, rotating the plates through an angle of 60° after each application [25]. The inoculated plates were allowed to dry for a few minutes at room temperature with the lid closed. The antibiotic discs were then placed aseptically on the inoculated plates using sterile forceps. Each disc was gently pressed down to ensure even contact with the culture media. The plates were incubated at 37°C for 24 hours. At the end of the incubation period, the results were recorded as sensitive or resistance based on the occurrence of zone of inhibition.

Determination of minimum inhibitory and bactericidal concentrations

Assessment of both minimum inhibitory and bactericidal concentrations of the plant extracts was conducted as described by Unachukwuet *al.* [24] and Ewansihaet *al.* [25] with minor modifications. Zero point six grams (0.6g) of each of the plant extracts were dissolved in 100ml w/v of distilled water to give 600mg/100ml which is equivalent to 6000 µg/ml. From the solution above, the desired concentrations such as 3000 µg/ml, 1500 µg/ml, 750 µg/ml, 375 µg/ml, 187.5 µg/ml, 93.75 µg/ml, 46.88 µg/ml, 23.45 µg/ml and 11.72 µg/ml were prepared by serial dilution in distilled water. A chain of test tubes containing Mueller Hinton broth **were** prepared in duplicates according to the number of the test concentrations for each of the plant extracts mentioned above and number of potential pathogenic bacterial isolates. **One milliliter (1ml)** of each of the different concentrations of crude n-hexane and ethanol extracts of the plants were mixed with the culture medium inside the test tubes. Serial dilutions of overnight cultures of the pathogenic bacterial isolates were made and each dilution was compared to a McFarland tube (0.5) equivalent to 1×10^8 cfu/ml. Following this, the broth/extract mixtures were inoculated with the test bacteria. The inoculated broths were incubated at 37°C for 24 hours. After 24 hours, the tubes were observed for growth and recorded as the minimum inhibitory concentration (MIC). The tubes with no growth after 24 hours were sub-cultured on freshly prepared Mueller Hinton Agar plates by the streaking method for the growth of potential bacteria pathogens. The culture media were incubated at 37°C for 24 hours and then observed for growth. After 24 hours, the lowest concentration from which the microorganisms did not recover and grow when transferred to the fresh media was recorded as the minimum bactericidal concentration (MBC).

Statistical Analysis of research data

Descriptive statistics, 2-way ANOVA and correlation analysis were used to statistically analyze the data generated from the laboratory investigations.

Results

Phytochemical composition of Lemongrass and Aloe vera

After phytochemical analysis of hexane and ethanol extracts of Lemongrass and Aloe vera plants, the phytochemical constituents identified and quantified in the extracts were alkaloid, flavonoid, **saponnin**, tannin, glycoside, steroid, phenol and terpenes as shown in Figure 1. The concentration of alkaloid recorded was higher in hexane and ethanol extracts of Aloe vera at 7.35mg/100g and 6.87mg/100g compared to the amount of alkaloid recorded for hexane and ethanol extracts of Lemongrass at 4.18mg/100g and 5.74mg/100g respectively. Flavonoid was higher in ethanol and hexane extracts of Lemongrass at 7.83mg/100g and 5.94mg/100g compared to the amount recorded for ethanol and hexane extracts of Aloe vera at 4.50mg/100g and 3.51mg/100g respectively. **Saponnin** was higher in hexane and ethanol extracts of Aloe vera at 2.75mg/100g and 2.24mg/100g than that recorded for hexane and ethanol extracts of Lemongrass at 1.85mg/100g and 1.41mg/100g respectively. Tannin was higher in hexane and ethanol extracts of Aloe vera at 10.23mg/100g and 8.59mg/100g compared to the amount of tannin recorded in hexane and ethanol extracts of Lemongrass at 5.39mg/100g and 3.05mg/100g respectively.

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Furthermore, higher concentration of glycoside was observed in ethanol and hexane extracts of Lemongrass at 3.68mg/100g and 2.52mg/100g compared to the amount of glycoside recorded in hexane and ethanol extracts of Aloe vera at 1.43mg/100g and 1.28mg/100g respectively. Higher concentration of steroid was found to be present in hexane and ethanol extracts of Lemongrass at 5.13mg/100g and 3.89mg/100g compared to the concentration of steroid recorded in hexane and ethanol extracts of Aloe vera at 2.91mg/100g and 2.34mg/100g respectively. Similarly, higher concentration of phenol was recorded to be present in ethanol and hexane extracts of Lemongrass at 24.54mg/100g and 29.9mg/100g compared to that recorded for ethanol and hexane extracts of Aloe vera at 17.36mg/100g and 13.52mg/100g respectively. Likewise, higher concentration of terpene was recorded in ethanol and hexane extracts of Lemongrass at 5.66mg/100g and 3.74mg/100g respectively.

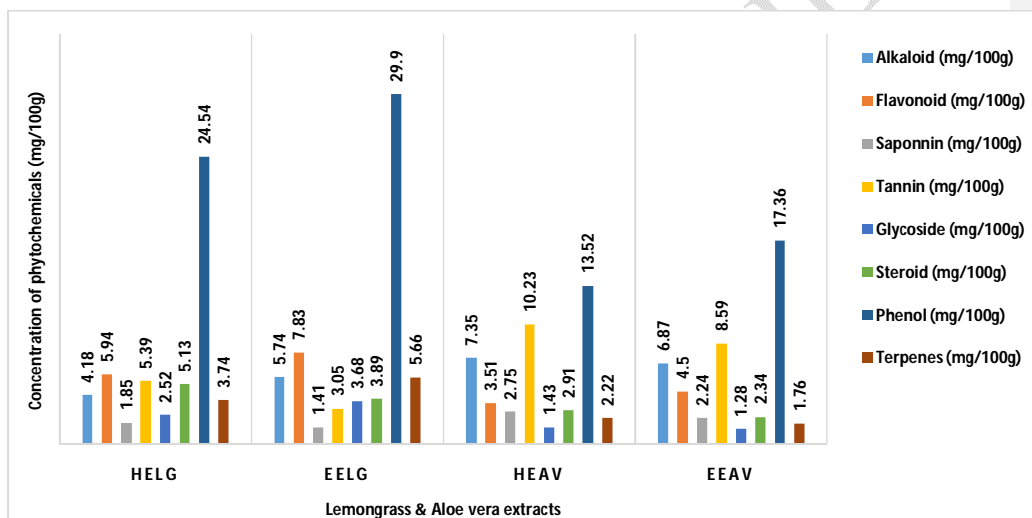


Figure 1: Concentration of phytochemicals detected in Lemongrass and Aloe vera extracts (Where; HELG = Hexane extract of Lemongrass; EELG = Ethanol extract of Lemongrass; HEAV = Hexane extract of Aloe vera; EEA V = Ethanol extract of Aloe vera).

Physicochemical properties of the water samples

Result of physicochemical properties of the surface and ground water samples analyzed are presented in [2]. Surface water samples collected from River Elioizu and Iwofe waterfront showed higher electrical conductivities of 164.3 $\mu\text{s}/\text{cm}$ and 138.3 $\mu\text{s}/\text{cm}$ compared to the ground water samples collected from Amino Kano (block A), Amino Kano (block B), King Jaja (block D) and King Jaja (block A), with electrical conductivities of 27.4 $\mu\text{s}/\text{cm}$, 26.8 $\mu\text{s}/\text{cm}$, 23.1 $\mu\text{s}/\text{cm}$ and 22.8 $\mu\text{s}/\text{cm}$ respectively (Fig. 2). Similarly, surface water samples obtained from River Elioizu and Iwofe waterfront showed higher turbidity of 3.35 NTU and 2.7 NTU compared to the ground water samples obtained from King jaja (block D), King

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jaja(block A), Amino Kano (block A) and Amino Kano (block B), with turbidity of 1.55 NTU, 1.50 NTU, 0.89 NTU and 0.88 NTU respectively.

Likewise, the concentration of nitrate was higher in samples of water collected from River Elioizu (17.45 mg/l) followed by Iwofe waterfront (14.6 mg/l), King Jaja block D (12.11 mg/l), King jaja block A (10.47 mg/l), Amino Kano block B (10.20 mg/l) and Amino kano block A (10.13 mg/l) respectively as shown in Figure 2. Furthermore, the concentration of phosphate in Iwofe waterfront, River Elioizu, Amino kano block B, Amino Kano block A, King Jaja block D and King Jaja block was 0.11 mg/l, 0.049 mg/l, 0.033 mg/l, 0.031 mg/l, 0.018 mg/l and 0.016 mg/l respectively. The surface water samples obtained from River Elioizu and Iwofe waterfront had higher BOD₅ of 6.78 mg/l and 5.92 mg/l compared to the ground water samples obtained from Amino Kano block A (3.18 mg/l), Amino Kano bloc B (3.16 mg/l, King Jaja block D (2.20 mg/l and King Jaja block A (2.12 mg/l) as shown in Figure 2. The pH of the surface water and ground water samples ranged from 6.60 to 6.36, while temperature of the samples ranged from 32.1°C to 30.0°C.

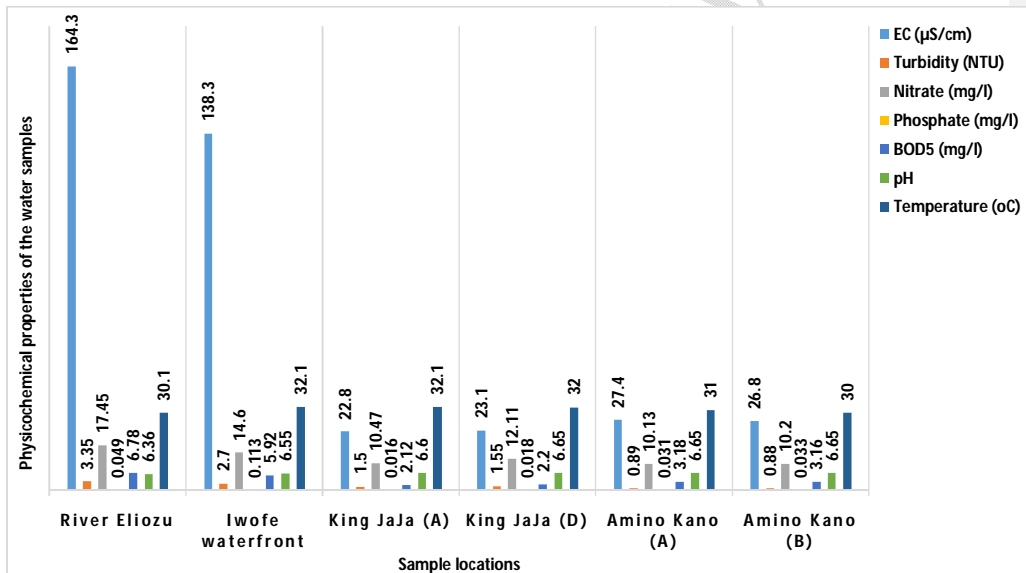


Figure 2: Physicochemical properties of water collected from the sample locations

Microbiology of the water samples

Bacterial counts (conducted on different types of agar media) for both surface and ground water samples obtained from the sample locations are presented in Figure 3. Surface water samples collected from River Elioizu and Iwofe waterfront had bacteria counts of 131.4×10^5 CFU/ml and 132.3×10^5 CFU/ml, 40.2×10^5 CFU/ml and 159.9×10^5 CFU/ml, 175.1×10^5 CFU/ml and 1.80×10^5 CFU/ml, 131.25×10^5 CFU/ml and 0 CFU/ml and 0 CFU/ml and 89.75×10^5 CFU/ml when incubated on the EMBA, MSA, MACA, SSA and CAB media respectively (Fig. 3). Bacteria counts for the ground water samples collected from King Jaja block A and D and Amino Kano block A were mostly absent or negligible. However, ground

water samples obtained from Amino Kano block B had bacteria counts of 7.5×10^5 CFU/ml, 12×10^5 CFU/ml, and 68.5×10^5 CFU/ml when incubated on EMBA, MACA and SSA respectively.

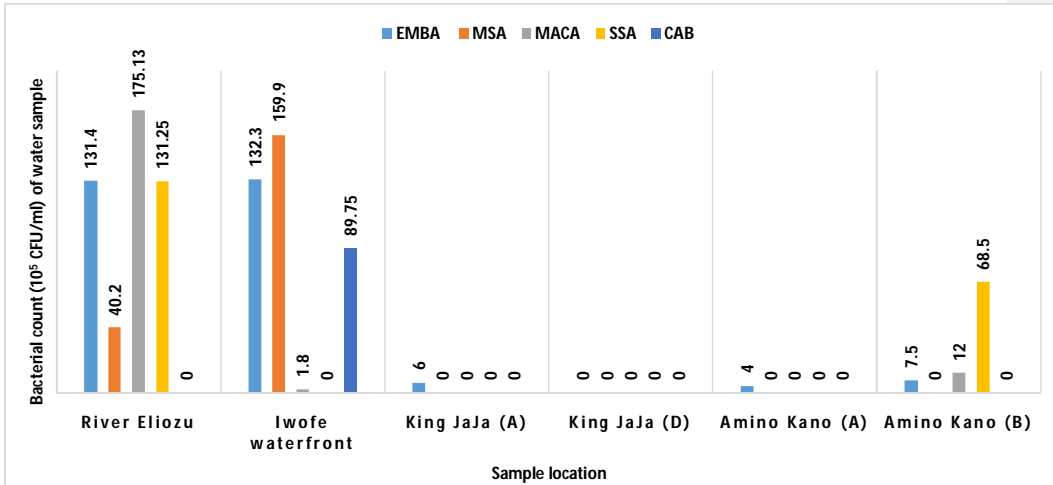


Figure 3: Bacteria counts of water samples collected from the sample locations

Figure 4 shows the bacteria isolated from the water samples were *Streptococcus* spp., *Shigella* spp., *Staphylococcus* spp. and *Pseudomonas* spp., which were considered potentially pathogenic and species of *Micrococcus*, *Bacillus*, *Seretia*, *Citrobacter*, *Providencia* and *Enterobacter* which were considered nonpathogenic.

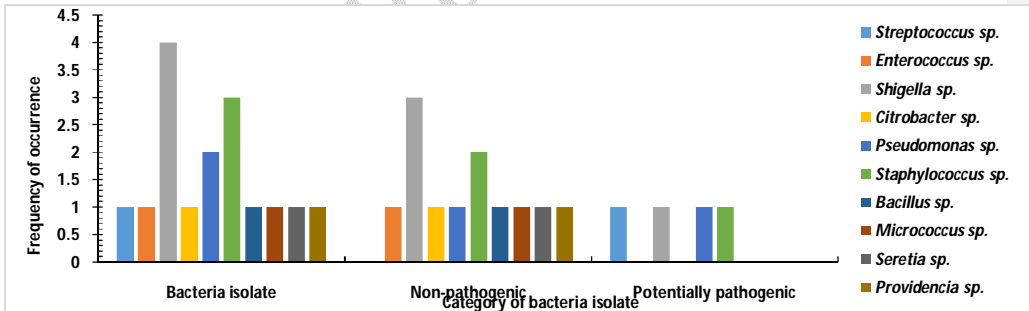


Figure 4: Bacteria isolated from water samples collected from the sample locations

Antibiotics susceptibility of the suspected bacterial pathogens

Result of the antibiotic susceptibility test for the suspected bacterial pathogens namely *Streptococcus* spp., *Shigella* spp., *Staphylococcus* spp. and *Pseudomonas* spp. is presented in Figure 5. All suspected bacterial pathogens were observed to be susceptible to hexane extract of the Lemongrass, with zones of inhibition which ranged from 10.2 mm to 14.5 mm. The result also showed that all suspected bacterial pathogens were susceptible to ethanol extract of the Lemongrass, with zones of inhibition which ranged from 16.7 mm to 20.9 mm. Hexane

extract and ethanol extract of the Aloe vera plant were also observed to be slightly active against all suspected pathogenic bacteria, with zones of inhibition which ranged from 4.2 mm to 9.5 mm and 6.7 mm to 11.2 mm respectively. Furthermore, some of the commercial antibiotics such as Ciproflox (CPX) and Streptomycin (STP) were observed to be active against all the suspected bacterial pathogens, with zones of inhibition ranging from 15.7 mm to 21.2 mm and 13.2 mm to 21.3 mm respectively. Other antibiotics such as Chloramphenicol (CHP) and Cuntamycin (CNT) showed slight activity against the suspected bacteria pathogens, with zones of inhibition which ranged from 3.1 mm to 14.5 and 3.3 mm to 14.7 mm respectively. Ofloxacin (OFX) and Augmentin (AUG) were observed to active against three of the four suspected bacterial pathogens namely *Shigella spp.*, *Staphylococcus spp.* and *Pseudomonas spp.*, with zones of inhibition which ranged from 11.8 mm to 19.9 mm and 5.4 mm to 12.2 mm respectively. Antibiotics such as Norfloxacin (NFX), Amoxil (AML), Rifampicin (RFC), Erythromycin (ERT), Ampiclox (APX) and Levofloxacin (LEV) showed activity against only one of the suspected bacterial pathogens (*Streptococcus spp.*), with zones of inhibition which ranged from 5.1 mm to 17.2 mm. *Shigella spp.* and *Pseudomonas spp.* were observed to be slightly susceptible to Peflacine (PEF), with zones of inhibition of 15.6 mm and 3.4 mm respectively. Ampicillin (AMP) showed weak and significant activity against *Staphylococcus spp.* and *Pseudomonas spp.*, with zones of inhibition of 3.1 mm and 18.0 mm respectively. Finally, Seprin (SEP) showed slight activity against the *Staphylococcus spp.*, with a zone of inhibition of 8.4 mm.

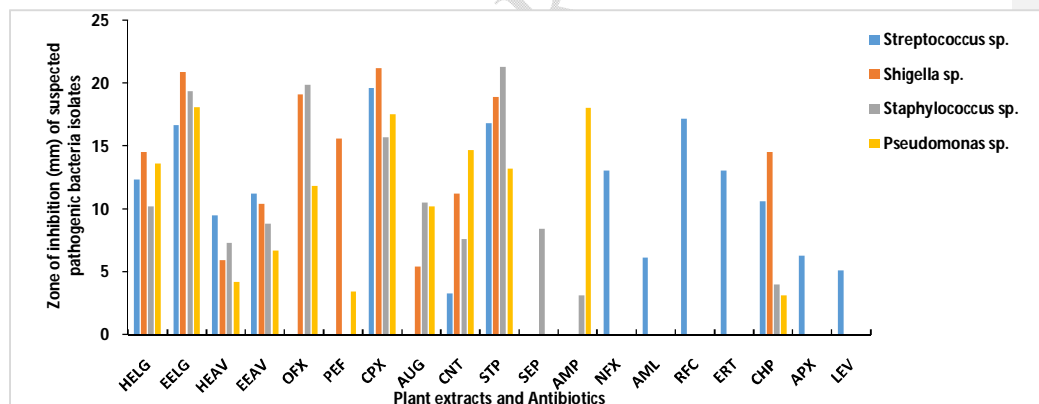


Figure 5: Antibiogram of suspected pathogenic bacteria isolated from the surface and ground water samples (OFX = Ofloxacin (10mg); PEF = Peflacine (10mg); CPX = Ciproflox (10mg); AUG = Augmentin (30 mg); CNT = Cuntamycin (10mg); STP = Streptomycin (30mg); SEP = Seprin (30mg); AMP = Ampicillin (30mg); NFX = Norfloxacin (10mg); AML = Amoxil (20mg); RFC = Rifampicin (20mg); ERT = Erythromycin (30mg); CHP = Chloramphenicol (30mg); APX = Ampiclox (20mg); LEV = Levofloxacin (20mg); HELG = Hexane extract of Lemongrass; EELG = Ethanol extract of Lemongrass; HEAV = Hexane extract of Aloe vera; EEA = Ethanol extract of Aloe vera; R (0) = Resistant).

MIC and MBC of Lemongrass and Aloe vera extracts

The MIC of hexane and ethanol extracts of lemongrass against the suspected bacterial pathogens namely *Streptococcus* spp., *Shigella* spp., *Staphylococcus* spp. and *Pseudomonas* spp., was 187.50 ug/ml and 93.75 ug/ml, 187.50 ug/ml and 93.75 ug/ml, 93.75 ug/ml and 93.75 ug/ml and 375.0 ug/ml and 187.50 ug/ml respectively (Figure 6). The MIC of hexane and ethanol extracts of Aloe vera against the *Streptococcus* spp., *Shigella* spp., *Staphylococcus* spp. and *Pseudomonas* spp. was 750.0 ug/ml and 375.0 ug/ml, 1500 ug/ml and 750 ug/ml, 1500 ug/ml and 1500 ug/ml and 3000 ug/ml and 1500 ug/ml respectively (Fig. 6).

Furthermore, the MBC of hexane and ethanol extracts of Lemongrass against the *Streptococcus* spp., *Shigella* spp., *Staphylococcus* spp. and *Pseudomonas* spp. was 750 ug/ml and 375 ug/ml, 750 ug/ml and 375 ug/ml, 375 ug/ml and 187.5 ug/ml and 1500 ug/ml and 750 ug/ml respectively. The MBC of hexane and ethanol extracts against the *Streptococcus* spp., *Shigella* spp., *Staphylococcus* spp. and *Pseudomonas* spp. was 6000 ug/ml and 1500 ug/ml, 6000 ug/ml and 3000 ug/ml, 6000 ug/ml and 6000 ug/ml and 6000 ug/ml and 6000 ug/ml respectively (Fig. 7).

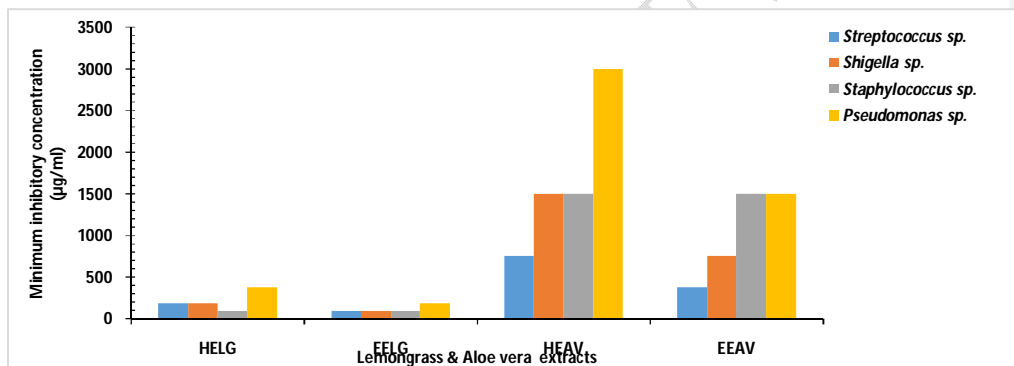


Figure 6: Minimum inhibitory concentration (MIC) of the Lemongrass and Aloe vera extracts (Where; HELG = Hexane extract of Lemongrass; EELG = Ethanol extract of Lemongrass; HEAV = Hexane extract of Aloe vera; EEAV = Ethanol extract of Aloe vera).

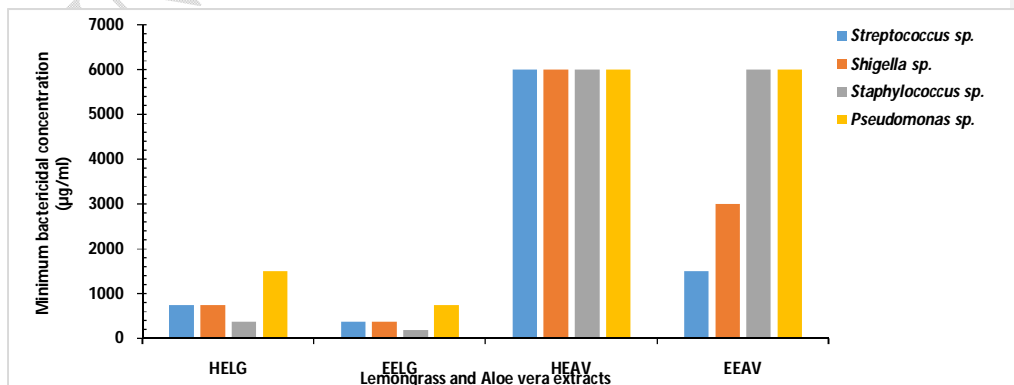


Figure 7: Minimum bactericidal concentration (MBC) of Lemongrass and Aloe vera extracts (Where; HELG = Hexane extract of Lemongrass; EELG = Ethanol extract of Lemongrass; HEAV = Hexane extract of Aloe vera; EEAV = Ethanol extract of Aloe vera).

There was a strong negative correlation between some of the phytochemicals (such as terpene, phenol, glycoside, flavonoid and steroid) and the MIC of the Lemongrass and Aloe vera extracts with respect to all four suspected bacterial pathogens. However, a strong positive correlation was observed between some of the other phytochemicals (such as tannin, saponin and alkaloid) and the MIC of the Lemongrass and Aloe vera extracts with regard to all four suspected bacterial pathogens (Fig. 8). Similarly, there was a strong negative correlation between some of the phytochemicals (such as terpene, phenol, glycoside, flavonoid and steroid) and the MBC of the Lemongrass and Aloe vera extracts with respect to all four suspected bacterial pathogens. However, a strong positive correlation was observed between some of the other phytochemicals (such as tannin, saponin and alkaloid) and the MBC of the Lemongrass and Aloe vera extracts with regard to all four suspected bacterial pathogens (Fig. 9).

Comment [s18]: Use the same style for figures, Fig. X or Figure X.

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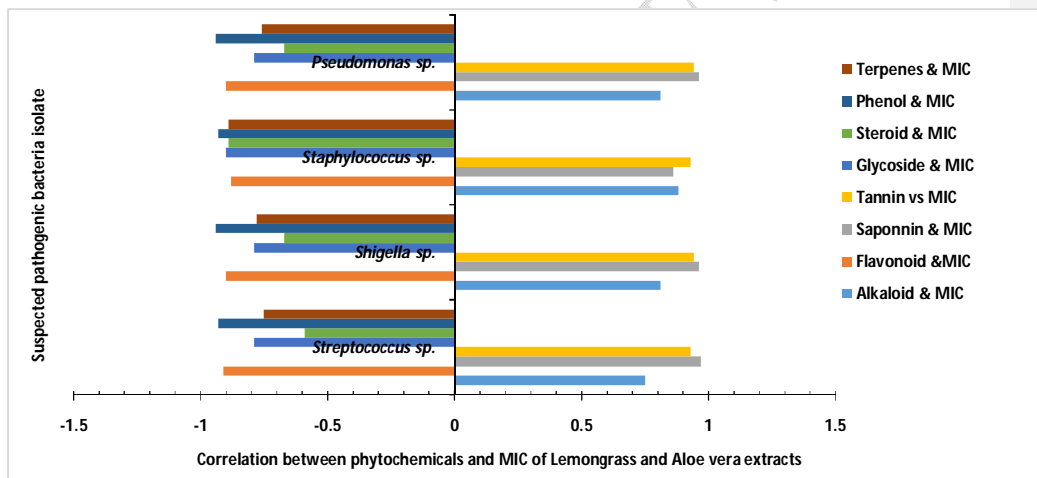


Figure 8: Relationship between the phytochemicals detected in Lemongrass and Aloe vera extracts and MIC of the extracts against suspected bacteria pathogens.

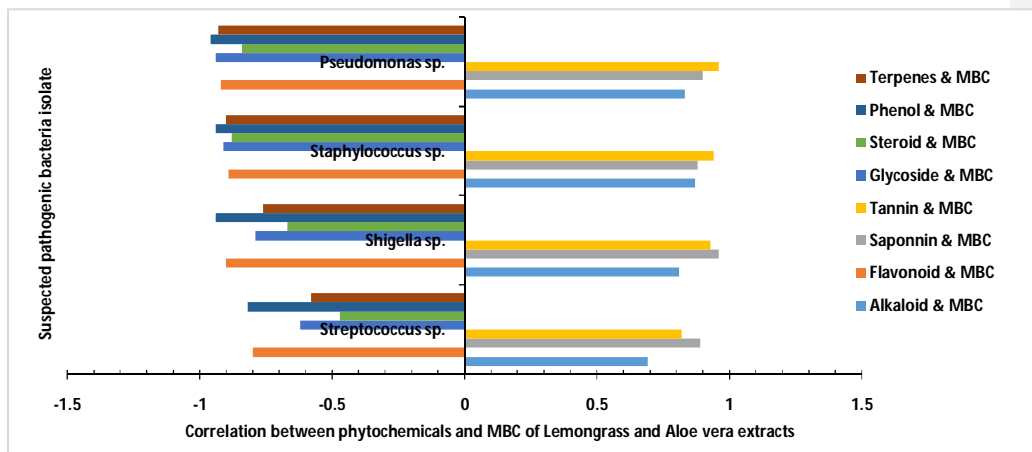


Figure 9: Relationship between the phytochemicals detected in Lemongrass and Aloe vera extracts and MBC of the extracts against suspected bacteria pathogens.

Discussion

Plants contain phytochemicals believed to be responsible for their numerous therapeutic functions. In the present study, phytochemicals such as terpene, phenol, glycoside, flavonoid, tannin, saponin and alkaloid and steroid were identified in hexane and ethanol extracts of Lemongrass and Aloe vera plants. The result above agrees with the findings of Ewansihaet *et al.* [25], Salome *et al.* [32], Mukherjee *et al.* [33], Dey *et al.* [34], Kar and Bera [35] and Jha *et al.* [36], who have also demonstrated the presence of these phytochemicals in both hexane and ethanol extracts of Lemongrass and Aloe vera respectively.

Two-Way ANOVA showed that there was no significant difference (at $p > 0.05$) between the concentrations of the phytochemicals detected in the Lemongrass and Aloe vera with respect to the method of extraction. However, there was a significant difference (at $p < 0.05$) in the concentrations of the phytochemicals extracted from both plants with respect to the nature of the phytochemicals themselves. This means that the type of extraction method did not significantly affect the quantity of phytochemicals extracted from the Lemongrass and Aloe vera plants. On the other hand, evidence from the 2-Way ANOVA result suggested that it was the nature of the phytochemicals which significantly affected the quantity of their extraction from both plants. This may be due to their individual ability to dissolve in the extraction solvents used in the present study [33-35].

The results for the physicochemical parameters of water samples showed some variations across location. For instance, electrical conductivities (ECs) of the surface water samples collected from River Elioizu and Iwofe waterfront were significantly higher than the ECs of the ground water samples obtained from King Jaja and Amino Kano hostels respectively. This indicates that higher amounts of impurities might have been present in the surface water samples compared to the ground water samples because water conductivity provides a

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measure of how much substances, chemicals and minerals are dissolved in the water samples. The higher turbidity of the surface water samples also appears to emphasize the fact that they may have contained higher amount of impurities compared to the ground water samples because it is an indication of the presence of higher amount suspended particles in the water bodies. Furthermore, the higher BOD recorded in the surface water samples when compared to the ground water samples indicated the presence of higher amount of putrescible organic matter in the surface water bodies. The amount of nitrate recorded in the surface water samples were also higher than those recorded in the ground water samples. Nevertheless, the amount of nitrate in the ground water samples was still a little high for human consumption. Higher concentrations of nitrates in drinking water can cause some serious health effects like weakness, excess heart rate fatigue and dizziness [37]. Finally, the result above showed that the ground water samples obtained from both King Jaja and Amino Kano hostels were cleaner than the surface water samples collected from River Eliozu and Iwofe waterfront.

Bacterial counts for surface water samples collected from River Eliozu and Iwofe waterfront ranged from 0 CFU/ml - 1.75×10^7 CFU/ml. Bacteria counts for the ground water samples collected from King Jaja block A and D and Amino Kano block A were mostly absent or negligible. However, ground water samples obtained from Amino Kano ranged from 7.5×10^5 CFU/ml – 6.85×10^6 CFU/ml. This result clearly showed that the bacterial load in the surface water samples obtained from River Eliozu and Iwofe waterfront was significantly higher than the bacterial load in the ground water samples that were collected from both King Jaja and Amino Kano hostels respectively. This may have resulted from that fact surface waters are more prone to all kinds of contamination from various sources (including faecal sources) because they are more exposed to the environment compared to ground waters, which can also be contaminated from same sources [38,39]. Furthermore, the higher turbidity, nitrate content and BOD recorded in the surface water samples indicated a possible contamination which might have contributed to their higher bacterial load compared to that of the ground water samples (Forstinuset *et al.*, 2016; Opeyemiet *et al.*, 2017).

In the present study, a total of 16 bacteria species belonging to genera *Streptococcus*, *Enterococcus*, *Shigella*, *Citrobacter*, *Pseudomonas*, *Staphylococcus*, *Bacillus*, *Micrococcus*, *Seretia*, and *Providencia* were isolated from the water samples as shown in Figure 5. Out of these bacteria isolates, four species namely *Streptococcus spp.*, *Shigella spp.*, *Pseudomonas spp.* and *Staphylococcus spp.* were identified to be potentially pathogenic due to their positive hemolytic reaction. Pathogens such as these have been isolated from both surface and underground water samples in previous studies and most of them can be conveyed through the water route [3, 40,41]

The result above showed that all four suspected bacterial pathogens (namely *Streptococcus spp.*, *Shigella spp.*, *Staphylococcus spp.* and *Pseudomonas spp.*) were susceptible to the crude hexane and ethanol extracts of the Lemongrass and Aloe vera plants with varying degrees. These results are in line with the observations made by Begum *et al.* [30], Nyarko *et al.* [42] and Kumar *et al.* [43], who also revealed the susceptibility of similar bacteria species to both hexane and ethanol extracts of Lemongrass and Aloe vera plants respectively.

Nevertheless, the suspected bacterial pathogens in the present study appeared to have shown higher susceptibility to the hexane and ethanol extracts of Lemongrass compared to the hexane and ethanol extracts of the Aloe vera. The antibacterial action of the crude ethanol extract of the Lemongrass on all four suspected pathogens was comparable to that of commercial antibiotics such as Ciproflox, Streptomycin and Ofloxacin which also demonstrated higher antibacterial action against the potential pathogens.

The antimicrobial activity results showed that both hexane and ethanol extracts of the Lemongrass and Aloe vera plants exhibited antibacterial activities against all four suspected bacterial pathogens (such as *Streptococcus* spp., *Shigella* spp., *Staphylococcus* spp. and *Pseudomonas* spp.) at various concentrations similar to the findings of Ewansihuet *al.* [25], Nyarko *et al.* [42] and Kumar *et al.* [43].

Two-way ANOVA showed that there was a significant difference ($p < 0.05$) in the susceptibility profile of the potential bacterial pathogens due to the variation in the type of antibiotics treatments they were exposed to. However, there was no significant difference ($p > 0.05$) in the susceptibility profile of the bacterial pathogens with respect to the variation in the nature of the bacteria species. This suggests that the variation observed in the rate of susceptibility of the potential bacterial pathogens was as a result of the different type of antibiotics they were exposed to and not significantly from the differences in the type bacteria species.

The MIC of hexane and ethanol extracts of the plants against the suspected bacterial pathogens ranged 93.75 ug/ml to 3000 ug/ml while the MBC of ranged from 375 ug/ml, 6000 ug/ml. In other studies, the MIC and MBC of both hexane and ethanol extracts of Lemongrass and Aloe vera plants against similar bacterial species ranged from 4 ug/ml to 6 ug/ml and 8 ug/ml to 10 ug/ml [44], 14 ug/ml to 32 ug/ml and 16 ug/ml to 38 ug/ml [25], 100 ug/ml to 2000 ug/ml and 200 ug/ml to 2,500 ug/ml [44]), 1,000 ug/ml to 4,000 ug/ml and 4,000 ug/ml [24], 100 ug/ml to 200 ug/ml and 200 ug/ml to 4,000 ug/ml [45] and 1,000 ug/ml to 8,000 ug/ml and 3,000 ug/ml to 10,000 ug/ml [46] respectively. Some of these values are comparable while others vary greatly. Nevertheless, in the present study, the results above showed that both hexane and ethanol extracts of the Lemongrass exhibited lower MICs and MBCs compared to the MICs and MBCs of hexane and ethanol extracts of the Aloe vera plant. This indicates that extracts of the Lemongrass plant performed better than extracts of the Aloe vera plant with regard to their antibacterial properties. Moreover, the ethanol extract of the Aloe vera exhibited a better performance than the hexane extract of the Aloe vera with respect to their antibacterial properties.

There was a strong negative correlation between some of the phytochemicals (such as terpene, phenol, glycoside, flavonoid and steroid) and the MIC of the Lemongrass and Aloe vera extracts with respect to all four suspected bacterial pathogens. However, a strong positive correlation was observed between some of the other phytochemicals (such as tannin, saponin and alkaloid) and the MIC of the Lemongrass and Aloe vera extracts with regard to all four suspected bacterial pathogens. Similarly, there was a strong negative correlation between some of the phytochemicals (such as terpene, phenol, glycoside, flavonoid and

steroid) and the MBC of the Lemongrass and Aloe vera extracts with respect to all four suspected bacterial pathogens. However, a strong positive correlation was observed between some of the other phytochemicals (such as tannin, saponin and alkaloid) and the MBC of the Lemongrass and Aloe vera extracts with regard to all four suspected bacterial pathogens.

This suggests that some of the phytochemicals (such as terpene, phenol, glycoside, flavonoid and steroid) identified in hexane and ethanol extracts of Lemongrass and Aloe vera may have significantly contributed more to the antibacterial properties of both plants compared to the other phytochemicals such as tannin, saponin and alkaloid respectively. This result seems to agree with the findings of other studies which showed that these phytochemicals identified in both plants in the current study, possess antibacterial properties to a varying degree [25,32,36,44].

Two-Way ANOVA showed that there was a significant difference (at $p < 0.05$) between the MICs of the Lemongrass and Aloe vera extracts due to the variation in the method of extraction of their respective phytochemicals. However, no significant difference (at $p > 0.05$) was observed between the MICs of the Lemongrass and Aloe vera extracts with respect to the variation in the suspected pathogenic bacteria species. Likewise, 2-Way ANOVA showed that there was a significant difference (at $p < 0.05$) between the MBCs of the Lemongrass and Aloe vera extracts due to the variation in the method of extraction of their respective phytochemicals. However, no significant difference was observed between the MBCs of the Lemongrass and Aloe vera extracts with respect to the variation in the pathogenic bacteria species.

These results suggest that the difference in the method of extraction significantly affected the antibacterial properties of the extracts of both plants irrespective of the type of bacterial species treated and the type of plants the phytochemicals were extracted from. These findings appear correlate with the results of Yi *et al.* [47] and Zhou *et al.* (2012) who also showed that the antibacterial properties of phytochemical extracts are largely dependent on the method of extraction from their parent plant if other factors were kept constant.

Conclusion

Phytochemicals such as terpene, phenol, glycoside, flavonoid, tannin, saponin and alkaloid and steroid identified in hexane and ethanol extracts of the Lemongrass and Aloe vera plants appeared to have contributed to the antibacterial properties of both plants against testorganisms. The extracts of the Lemongrass plant performed better than extracts of the Aloe vera plant with regard to their antibacterial properties.

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Comment [s22]: Please, mention something about the potential use of these extracts to control pathogens found in water sources.

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