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Assessment of Disinfection Efficacy of Sodium Hypochlorite and Aloe Vera Gel for Potable Water Production from Multiple Water Sources

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Abstract

The importance of potable water for human survival cannot be understated, just as human body cannot carry out its functions without water. Oftentimes, chemical such as sodium hypochlorite is used to make contaminated water fit for human consumption but overuse of this chemical has its attendant health effects including carcinogenic effect. It is therefore, important to find a substitute for sodium hypochlorite, without attendant health effects, for water disinfection purpose. The aim of this study was to evaluate and compare the antimicrobial effects of sodium hypochlorite (NaOCl) and aloe vera gel, stored under various conditions and parameters, on coliform and *Escherichia coli* isolates from some water samples. Four water samples including tap water, well water, oil polluted water and leachate were collected across Lagos State, Nigeria. All water samples were subjected to presumptive, confirmatory and complete tests. Disinfectant efficacies of the NaOCl and aloe vera were evaluated and compared on the eight isolated microorganisms from the water samples, using the disc diffusion method with various condition and parameters for the two disinfectants. The results showed that coliform counts were <2, 49, >1800 and >1800 cfu/100ml for the tap water, well water, oil polluted water and leachate respectively. Aloe vera looks more stable in its disinfectant capability when compared with NaOCl at the storage temperatures especially at 0, 25 and 35^{o}C. Furthermore, the p-values for using aloe vera and NaOCl as disinfectants under various conditions of concentration, temperature and sun exposure period ranged between 0.011 and 0.749 with most of the p-values indicating non-significant differences while few ones showed significant differences. On the average, the results showed that aloe vera could be used as a substitute for sodium hypochlorite for disinfection of water from multiple sources.

Keywords: coliforms; aloe vera; sodium hypochlorite; potable water; disinfectants**.**

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1. Introduction

The inevitability of water as a sustenance of life is undeniable as human body cannot carry out its functions without water [1].While earth's surface is covered with much water, potable water is not always available where and when humans need it since existing freshwater resources are being polluted by human activities [2, 3]. Water bodies are contaminated by substances including heavy metals, dyes; pharmaceuticals; pesticides, fluoride, phenols, insecticides, pesticides, detergents, as well as, pathogenic microorganisms [2]. Water contamination may be geological or anthropogenic; the natural occurring elements present at unacceptable levels can contaminate water while other man-made by-products of industry, and agriculture, including heavy metals like mercury, copper, chromium, lead, and hazardous chemicals, dyes and compounds like insecticides and fertilizers, can also contaminate water as well [4-7]. Contaminated water may have adverse effects on human health and aquatic ecosystems; it is, therefore, important that effective wastewater treatment is carried out on polluted water to ensure that water that people drink or use domestically is free of germs and toxic chemicals. Different techniques including coagulation, membrane process, adsorption, dialysis, foam flotation, osmosis, photocatalytic degradation and biological methods, among others, have been used for the removal of toxic pollutants from contaminated water [8]. However, application of these techniques have been limited by many factors, such as processing efficiency, energy requirement, engineering expertise, economic benefit and infrastructure, among others. Disinfection methods of waste water treatment including ozonation and chlorination have been introduced to control human pathogens in water, however, the efficacy of disinfection is dependent on various factors such as the organic load present, the type and level of microbial contamination, the disinfectant concentration and exposure time of the microbial contaminants to the disinfectant, the temperature and pH of the disinfection process, among others [9, 10]. Microbial contaminants include pathogens like bacteria, viruses, and parasites such as microscopic protozoa and worms. Some of these contaminants can be easily identified by assessing color, odor, turbidity and the taste of the water. However, most cannot be easily detected and require testing to reveal whether the water is contaminated or not. During disinfection of water using disinfectant chemical reagents (chlorine gas, chloride dioxide, ozone, chloramines, sodium hypochlorite, calcium hypochlorite and lithium hypochlorite), harmful halogenated disinfection byproducts (DBPs) are formed [10-12]. The challenge of formation of undesirable disinfection by-products; as well as, the instability of the chemical disinfectants when stored at higher concentrations, lower pH, higher temperature and where it is exposed to sunlight could affect the disinfection efficiency in terms of quality of the treated water and process economy. Meanwhile, plants including aloe vera, known to have almost limitless ability to synthesize aromatic substances that are effective against viruses, bacteria, and fungi [13] are being proposed as substitutes for the chemical disinfectants in order to overcome the challenges associated with their usage. Some of these plants that have profound antimicrobial or disinfectant properties include Azdirachta indica, Eucalyptus robusta, Aloe barbadensis, Aloevera, Withania somniferum, Andrographis paniculata, Aegle marmelos, Berberis vulgaris, Cinnamomum verum, Piper nigrum, Rhamnus purshiana, Capsicum annuum, Syzygiumaromaticum, Eucalyptus globulus, Gaultheria procumbens, Cassia angustifolia, Cassiafistula, Mentha piperita, among others[14]. Antimicrobial effect of aloe vera for being able to target the bacterial cell wall and membrane is due to its main gel components which are anthraquinones, phenols and terpenoids [15, 16]**.** The effectiveness of aloe vera as disinfectants is however dependent on factors such as temperature, pH and organic matter, among others, of the

aloe vera medium [17]**.** Quite a lot of research studies have been conducted on water disinfection but few literatures exist on using plants as disinfectants for water disinfection. In a study conducted by Trivedi and his colleagues [18], Aloe vera was used as natural alternative for disinfecting dental impression materials. The efficacy of the disinfection procedures was assessed by determining the number of colony-forming units (CTU) recovered after disinfection with aloe vera solution (99.96%). It was found that there was a mean percentage reduction in colony count of Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans after 3 minutes of immersion in aloe vera and after 3 minutes spray disinfection. Other research studies that investigated the usage of aloe vera as water treatment agent included aloe vera usage as; coagulants or flocculants [19-28], and bisorbent [29-36]. In another study conducted by Pareek and his colleagues [37] on disinfection of dental unit water line using Aloe Vera, the comparison was made on the efficacy of aloe vera, 5% sodium hypochlorite (NaOCl), and 10% hydrogen peroxide (H_2O_2), each at different concentrations, in controlling microbial contamination of dental unit water system. However, effect of temperature and variation in concentration were not evaluated on the disinfectants used. Therefore**,** the aim of this study was to compare the antimicrobial effects of sodium hypo chlorite and aloe vera gel, stored under various conditions, for the purpose of controlling microbial contamination of water samples. The study will be useful to determine the suitability of using Aloe vera gel as a substitute for sodium hypochlorite disinfectant. Application of aloe vera gel for water disinfection may help in the effort to overcome the challenges of formation of undesirable disinfection by-products and the instability of chemical disinfectants when stored at higher concentrations, higher temperature and where it is exposed to sunlight. Besides, resolving the challenges can improve the effectiveness of the disinfection process of water which, as a consequent, can affect the process economy and the quality of the treated water. The scope of this research work was limited to the coverage of only *E coli* indicator as a test for microbial water contamination since *E. coli* is the most important indicator used in drinking water quality testing. It is a thermo-tolerant coliform bacteria found mainly in faeces of warm-blooded animals, including humans. The study did not cover other microorganisms that might be present in water. Besides, only four sources of water were used as samples in the study. Other sources of water including rivers, oceans, rainfall, among others were not considered.

2. Methodology

2.1. Materials

The materials used during the research work were sample bottles with stoppers, aluminium foil, autoclave, distilled water, gas burner, alcohol solution, $Na_2S_2O_3{\cdot}5H_2O$ (1g/L), Mac Conkey broth (Purple), Kovacs reagent, Mac Conkey Sorbitol Agar Base, crude oil, heating mantle, magnetic stirrer, pipettes: (0.1, 1ml and 10 ml), test tubes and racks, media preparation equipment (glass containers), gas burner, culture tubes containing inverted vials (Durham tubes), inoculation loop and holder, safety equipment (fire extinguisher), culture medium, incubator (s) or water-baths, and balance. In this study, four water samples from different sources were used including tap water from Lagos State University water plants with a geographical location of 6.47043 N and 3.20253E, leachate from Solous dumpsite at Igando in Alimosho local government area, Lagos with a geographical location of 6.56497 N and 3.25131 E, well water sample from Isheri Osun in Isolo local government area, Lagos with a geographical location of 6.53872 N and 3.25131 E and oil polluted water sample from Ibafon in Apapa local government area, Lagos with a geographical location of 6.435122 N and 3.315168

E.

2.2. Experimental procedure

The experimental work included preparation and sterilization of sample bottles, collection of water samples, disinfectants preparation and microbiological analysis of water, as well as, disc diffusion method to test for the disinfectant efficacy, Different storage conditions of disinfectants that could possibly affect the effective disinfection of water samples from multiple sources were examined. The storage disinfectant conditions included disinfectant concentration, disinfectant temperature and disinfectant exposure period to sunlight.

2.2.1. Preparation and sterilization of sample bottles

During the preparation and sterilization of sample bottles, the sample bottles were washed thoroughly and rinsed with distilled water. Screw bottle caps were loosely fastened to the sample bottles prior to sterilization in an autoclave. A strip of aluminium foil was placed inside the neck of each sample bottle to prevent the stopper from getting stuck during sterilization. The stopper was used to protect the sample bottles from contamination. The steam sterilization of the bottles was done at 120ºC for 20 minutes. After the sterilization, the bottles were allowed to cool before they were tightened using the stoppers and then stored in a refrigerator until required.

2.2.2. Collection of water samples

Collection of water samples was done using standard procedure. To draw sample water from tap water, the hands were washed and then the tap was cleaned. The hands were washed again before opening the tap. The tap was then opened until it reached its maximum rate of flow. The water was allowed to flow from the tap for 1 to 2 minutes to clear the service line. The tap was then closed so that the tap spout could be sterilized by using a solution of alcohol since the spout was made of plastic. After the spout was sterilized, the tap was opened again to allow the water to flow for 1 to 2 minutes at normal rate. The sterilized bottle was then opened carefully (while keeping the fingers on the aluminum foil) and immediately put under the water jet for the bottle to be filled to the shoulder with approximate volume of 100mL of tap water. After filling the sample bottle to the shoulder, five drops of aqueous 1g/L of sodium thiosulphate ($Na_2S_2O_3 \cdot 5H_2O$) were added to neutralize any residual chlorine which could distort the results of the analyzed sample. The tap was turned off and stopper, along with the aluminium foil, was used to tighten the sample bottle. The bottle was then gently shaken to allow uniform mixing of the content. A label, containing the location, the time of sampling, the date and the sampler's name, was attached to the sample bottle. The sample bottle was stored in melted ice and transported to the laboratory for analysis to be carried out within two hours. To draw sample water from a well fitted with a pump, the pump was operated for 20 minutes to clear any standing water in the water column. The hands were washed and then pump outlet was cleaned thoroughly. The same process as described for sampling from a tap was then followed. Regarding the sampling from a leachate flow, the hands were thoroughly washed, and then the sample bottle cap was carefully opened without touching the neck of the bottle, to avoid possible contamination. The sample bottle was held by the base and submerged into the leachate flow with the depth of about 30 cm while tilting the bottle neck slightly upwards to let it full completely. After the bottle was completely full, some of the

content was discarded so that the sample could be shaken before analysis. Five drops of aqueous 1g/L of sodium thiosulphate ($Na_2S_2O_3 \cdot 5H_2O$) were added to neutralize any residual chlorine which could distort the results of the analyzed sample. The bottle cap, along with the aluminum foil, was carefully used to tighten the sample bottle without touching the neck of the bottle. The bottle was then gently shaken to allow uniform mixing of the content. A label, containing the location, the time of sampling, the date and the sampler's name, was attached to the sample bottle. The sample bottle was stored in melted ice/refrigerator and transported to the laboratory for analysis to be carried out within two hours. For the oil polluted water, simulated crude oil contaminated water sample was prepared by adding crude oil to water in ratio 1:10 in a container. The mixture was heated and stirred for about 1 hour to ensure homogeneous mixture at a temperature of 30°C. The crude oil used was obtained from Nigerian refinery while the water used was from nearby stream river. To draw a sample from the simulated crude oil contaminated water in a container, the same procedure that was carried out when sampling from a leachate flow, was followed [38]. Care was taken to ensure that plastic, rubber or *similar materials including gloves, bags or cleaning solvents, did not come in contact with the sample.*

2.2.3. Preparation of disinfectants

Two disinfectants including sodium hypochlorite and aloe vera gel, were prepared for the experimental work. 5% sodium hypochlorite was obtained from the market and diluted to obtain four more concentrations (1, 2, 3, and 4%). The different concentrations were stored under different conditions. Regarding aloe vera, full grown plants were obtained and cut open to extract the gel from the plant. 5g of the extracted gel was then dissolved in 95 g of distilled water to obtain 5% aloe vera solution from which several dilutions were made to obtain four other concentrations (1, 2, 3, and 4%).

2.2.4. Microbiological analysis of water samples

Most probable number (MPN) / multiple-tube method was the microbiological analysis that was used in this research because some of the water samples (leachate and oil-polluted water) are turbid. MPN test was performed in three steps which include presumptive test, confirmatory test and completed test. The presumptive test was used to screen, detect and estimate water samples total coliform organisms that fermented lactose at 35–37ºC within 24 – 48 h. MacConkey broth (Purple) that contained indicator Bromocresol purple was the culture medium used for the presumptive detection and isolation of coliform organisms in the water samples. Confirmatory test was therefore done in order to confirm the presence of faecal thermo-tolerant coliforms (*E.coli*): that ferment lactose at 44 – 45º C. The completed test was used to isolate enteropathogenic *E.coli* O157:H7 from other strains of *E.coli* using a differential Sorbitol MacConkey Agar. The Sorbitol MacConkey Agar medium was used to isolate *E.coli* O157:H7 an enteric pathogen by the presence of colourless circular colonies that are non-sorbitol fermenting. The number of total coliforms was determined by counting the number of tubes giving positive reaction (Plates 1 & 2). From the number of positive tubes the most probable number of coliform bacteria present in 100 ml of the original water was estimated by reference to standard statistical tables. The results were reported as CFU/ 100 mL

2.2.5. Disc diffusion method for the disinfectant efficacy

The disc diffusion method was used to examine the susceptibility of the isolated coliforms to disinfectant activities of sodium hypochlorite and Aloe Vera [39]. Nutrient agar medium was used for growth inhibition. After media sterilization at 121^{o}C for 15min and 15psi, plates were left overnight at 37 ± 1^{o}C for pre-incubation. Freshly grown bacteria were seeded on the medium plates to grow bacterial colonies. Stock solutions of sodium hypochlorite NaOCl and Aloe vera gel with a pH of 12.8 and 4.3, respectively, were subjected to varying conditions including varying concentration, storage temperature and period of exposure to sunlight in order to investigate the treatment condition under which NaOCl and Aloe vera gel will be most effective in disinfecting the isolated microorganisms. The sodium hypochlorite and Aloe Vera impregnated discs were then placed on the surfaces of the inoculated agar plates. The plates were inverted and incubated at 37 ± 1^{o}C for 24 h to see the effect of disinfectants (NaOCl and Aloe vera) under various conditions (concentration, storage temperature, exposure time to sunlight) on microbial growth. Plate 3 shows the media for the disinfectant activities investigation while Plate 4 shows an example of the different zones of inhibition observed after 24 hours of incubation

3. Results and Discussion

3.1. Results

3.1.1. Multiple tube fermentation tests.

The contamination of water samples by bacteria was investigated using multiple tube fermentation method. The presumptive, confirmatory and completed tests showed and confirmed the presence of coliform bacteria in water samples from three sources including well water, oil polluted water and leachate (Tables 1 & 2). The well water contained faecal thermo-tolerant coliform (non - *E coli*); the oil polluted samples contained faecal thermotolerant *E. coli,* pathogenic faecal *E coli and* non- pathogenic faecal *E coli,* the leachate contained faecal thermotolerant non- *E coli* and faecal thermo-tolerant *E. coli,* as well as, non-pathogenic faecal *E coli* and pathogenic faecal *E coli*

3.1.2. Disc diffusion method for the disinfectant efficacy on the isolated coliforms

The disinfectant efficacy of the NaOCl and Aloe vera was evaluated and compared on the eight isolated microorganisms from the three water samples using the disc diffusion method with various condition and parameters for the two disinfectants. The results showed different degree of inhibition of the disinfectants against the isolates from various water samples under different conditions and parameters. The inhibition zones for NaOCl and Aloe

Plate 1: Presumptive test: showing the result after 48 hrs incubation within 24–48 h.

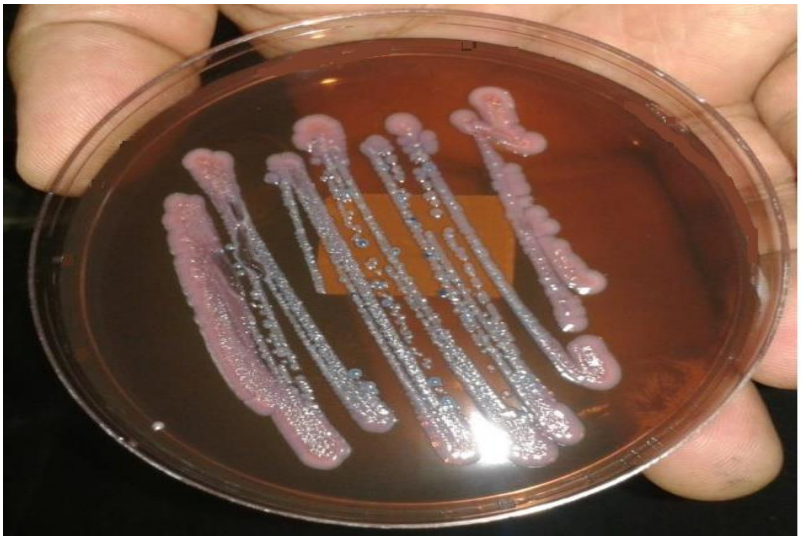

Plate 2: Complete test showing growth of both pathogenic and non-pathogenic.

E. coli on MacConkey sorbitol after 24hrs incubation at 37^{o}C

Plate 3: Plates with Nutrient agar medium (NAM) used for disc diffusion with different parameters of the disinfectants i.e. NaOCl and Aloe vera gel

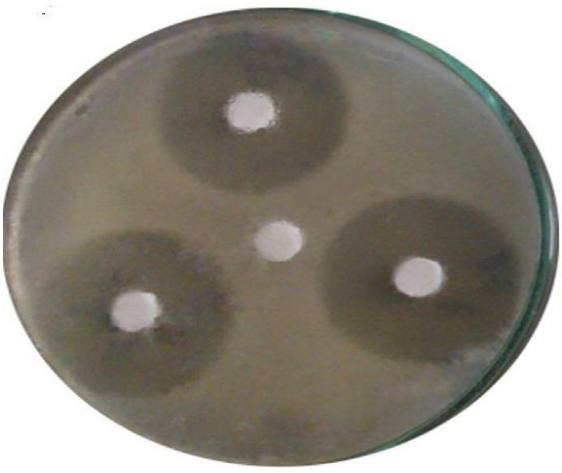

Plate 4: A plate showing cleared zones of inhibition of the disinfectant against the isolated coliform.

Table 1: Physical parameters and microbiological analysis of water samples from multiple sources.

S/N	Water samples	Colour	Turbidity (NTU)	Number of positive tubes after 48 hrs			Coliform count (CFU/100ml)	95% Confidence limits	
				10ml	1ml	0.1ml		Lower	Upper
1	Tap water	Colourless	4	0	0	0	< 2	<1	7
2	Well water	Colourless	12	5	2	9	49	17	120
3	Oil polluted water	Light brown	90	5	5	5	>1800	-	-
4	Leachate	Dark brown	211	5	5	5	>1800	-	-
5	WHO*	Colourless	25				0 per 100ml		
6	NAFDAC **	Colourless	5				10 per 100ml		

WHO: World Health Organization;

NAFDAC: National Agency for Food and Drug Administration and Control

Table 2: Sources of water and their composition of coliform and Eschericia coli.

S/N	Water samples	Confirmatory Test		Completed Test	
		Thermo-tolerant Coliform (non E-coli)	Faecal Thermotolerant E-coli	Pathogenic Faecal E-coli	Non-pathogenic faecal E-coli
1	Tap water	Negative	Negative	Negative	Negative
2	Well water	Positive	Negative	Negative	Negative
3	Oil polluted water	Negative	Positive	Positive	Positive
4	Leachate	Positive	Positive	Positive	Positive

vera gel under these varying conditions for well water, oil polluted water and leachate are shown in Tables 3, 4 and 5, respectively

3.2. Discussion

The results of microbiological analysis of water samples from four water sources indicated that only tap water had a coliform count value that was within the World Health Organization (WHO) [40] and National agency for Food and Drugs Administration and Control (NAFDAC). The well water showed a coliform count of 49 CFU/100ml. Amaeze and Irekeola [41]; Akinyemi and his colleagues [42] reported contamination of well water used in their studies with one or more bacterial pathogens. The oil polluted water sample and leachate showed a very high value of coliform count >1800 CFU/ml. The confirmatory test carried out in this study showed that the coliform present in the well water sample was thermotolerant coliform that are non- *E. coli*. The Oil polluted water sample showed the presence of Faecal thermo tolerant *E. coli*, pathogenic Faecal *E. coli* and non-pathogenic *E. coli*. On the other hand, thermo-tolerant coliform that were non-*E. coli*, *f*aecal thermo tolerant *E.*

coli, pathogenic faecal *E. coli* and non-pathogenic *E. coli* were present in the leachate sample investigated in this study. Regarding the concentration of the disinfectants, 5% storage concentration of NaOCl solution showed high clearance than all other storage concentrations (4%, 3%, 2%, 1%) in the disc diffusion test, carried out to investigate the disinfectant capabilities of NaOCl against the isolated coliforms in the three wates samples. In the study, the NaOCl at all storage temperature i.e. 0, 25, 35, 45 and 55^{O}C showed clearance ranging from 1-5mm in diameter. At storage temperature 0, 25, and 35^{O}C, high value of clearance was observed when all storage temperatures were compared in terms of degree of clearance. del Carpio-Perochena *and his colleagues* [43] also reported that 25 and 35^{o}C NaOCl were effective in dissolving the bacteria biofilm without significant differences between them. In the study by Sirtes and his colleagues [44] using *E. faecalis* cells; a temperature raise of 25°C increased NaOCl efficacy by a factor 100 and of which the same was experienced in this study when compared to the increase in temperature from 25^{O}C to 55^{O}C with the efficacy of NaOCl at 150% on faecal pathogenic *E. coli* in oil polluted water and faecal thermo-tolerant *E. coli* in leachate. Moreover, in this study, NaOCl increased in their disinfectant efficacy with the increase in number of hours they were exposed to sunlight. The NaOCL exposed to 10 hrs of sunlight had a clearance of 3-12 mm in diameter across all the isolates in this study compared to the 0 hrs and 4 hrs exposed NaOCl which had clearance diameters of 1-5 mm and 1-3 mm respectively. Meanwhile, Aloe Vera liquids have shown to have a wide range of effectiveness against Gram positive and Gram negative bacteria [16, 37]. The plant's antimicrobial agents were reported to effectively inhibit the growth, greatly reduce or kill several coliform bacteria such as *E. coli*, *Enterobacter aerogenes*, *Enterococcus faecalis, Klebsiella*.

Table 3: Antimicrobial sensitivity of the well-water Isolated Organism to Sodium hypochlorite and Aloe vera disinfectants.

S/N	Disinfectant Treatment	Parameter	Zone of growth inhibition of FTC by NaOCl	Zone of growth inhibition of FTC by Aloe vera	P-values for FTC
1	Storage Concentration (%)	1	1	3	1.000
		2	4	3	
		3	2	3	
		4	5	4	
		5	6	5	
2	Storage Temperature (ºC)	0	4	6	0.468
		25	4	6	
		35	5	6	
		45	1	1	
		55	2	0	
3	Exposure period to sunlight (hr)	0	1	5	0.247
		4	3	0	
		6	4	1	
		8	5	2	
		10	12	2	

FTC: Faecal Thermo-tolerant Coliform (non-Ecoli)

Table 4: Antimicrobial sensitivity of oil polluted water isolated organisms to sodium hypochlorite and aloe vera disinfectants.

			Faecal Thermotolerant E.coli (FTE)			Pathogenic Faecal E.coli (PFE)			Non- Pathogenic Faecal E.coli (NFE)		
S/N	Disinfectant Treatment	Parameter	Zone of growth inhibition of FTE by NaOCl (mm)	Zone of growth inhibition of FTE by Aloe vera (mm)	P-values for FTE	Zone of growth inhibition of PFE by NaOCl (mm)	Zone of growth inhibition of PFE by Aloe vera (mm)	P-values for PFE	Zone of growth inhibition of NFE by NaOCl (mm)	Zone of growth inhibition of NFE by Aloe vera (mm)	P-values for NFE
1	Storage Concentration (%)	1 2 3 4 5	1 2 4 4 5	3 2 3 2 5	0.778	2 2 3 4 4	1 1 1 1 1	0.011	2 2 3 4 4	2 3 2 2 5	0.749
2	Storage Temperature (ºC)	0 25 35 45 55	5 5 1 2 1	0 5 0 0 0	0.105	2 2 3 2 5	1 1 2 3 4	0.208	4 4 2 2 2	5 4 4 4 5	0.035
3	Exposure period to sunlight (hr)	0 4 6 8 10	1 3 3 3 4	4 0 0 0 0	0.189	2 3 2 4 4	1 4 5 3 8	0.305	2 3 3 4 6	2 2 2 3 5	0.016

FTE: Faecal Thermotolerant E.coli; Pathogenic Faecal E.coli (PFE); NFE: Non- Pathogenic Faecal E.coli

Table 5: Antimicrobial sensitivity of leachate isolated organisms to sodium hypochlorite and aloe vera disinfectants

			Faecal Thermo-tolerant non-*E coli* Coliform (FTC)			Faecal ThermotolerantE.coli (FTE)			Non- Pathogenic Faecal E.coli (NFE)			Pathogenic Faecal E.coli (PFE)		
S/N	Disinfectant Treatment	Para-meter	Zone of growth inhibition of FTC by NaOCl	Zone of growth inhibition of FTC by Aloe Vera	P-values for FTC	Zone of growth inhibit--ion of FTE by NaOCl	Zone of growth inhibit-ion of FTE by Aloe Vera	P-values for FTE	Zone of growth inhibit-ion of NFE by NaOCl	Zone of growth inhibit-ion of NFE by Aloe Vera	P-values for NFE	Zone of growth inhibition of PFE by NaOCl	Zone of growth inhibition of PFE by Aloe Vera	P-values for PFE
1	Storage Concentration (%)	1	2	2	0.135	2	2	0.129	2	2	0.047	1	1	0.033
		2	2	4		2	3		2	0		1	2	
		3	3	7		3	4		4	3		1	2	
		4	4	5		4	6		5	1		1	3	
		5	5	5		2	8		5	2		1	3	
2	Storage Temperature (ºC)	0	1	10	0.006	1	9	0.305	5	5	0.242	3	3	0.587
		25	2	10		2	5		5	5		1	3	
		35	3	9		2	3		2	5		4	3	
		45	2	6		3	3		2	3		3	2	
		55	2	5		5	5		3	3		3	1	
3	Exposure period to sunlight (hr)	0	1	5	1.000	1	1	0.208	5	1	0.646	1	1	0.016
		4	2	2		2	2		2	3		1	2	
		6	3	2		3	3		1	4		1	2	
		8	4	2		4	3		2	4		2	3	
		10	4	3		5	3		4	5		3	4	

FTE: Faecal Thermotolerant E.coli; FTC: Faecal Thermotolerant Coliform(non-Ecoli); PFE: Pathogenic Faecal E.coli NFE: Non- Pathogenic Faecal E.coli

pneumonia [45]. In this study, Aloe Vera gel display disinfectant abilities in the various storage concentrations ranging from 1% to 5% on the isolated organism from the three different water samples used in this study. The 5% storage concentration displayed clearance of 1-8mm in diameter while the 1% displayed 1-3 mm diameter of clearance around the disc impregnated with the gel. The faecal non-pathogenic *E. coli* from the leachate sample showed a resistance to 2% of the storage concentration of the aloe vera [37]. In this study, at a storage temperature of 0 and 25^{o}C, the aloe vera gel was able to show its disinfectant efficacy by 1-10 mm zone of inhibition respectively with growth of the isolated microorganism. Also at a storage temperature of 35^{o}C, the aloe vera gel was able to show its disinfectant efficacy by 2-9 mm zone of inhibition.

In the study by Kusuma and his colleagues [46], all formulas of aloe vera gel at a storage temperature of 18°C produced a higher diameter of inhibition than at 25°C against *Staphylococcus aureus*. Besides a synergic action between different components in aloe vera gel, some polysaccharides characteristic seem to play roles in its pharmacological and physiological [47]. But the improper storage and length of time for storing herbal products could lead to degradation processes which could cause reduction in active substances resulting in metabolites with no activity, and the most extreme is the forming of toxic metabolites [48]. In this study, faecal thermo-tolerant isolated from oil polluted water showed resistance to the aloe vera gel at the storage temperature except at 25°C where 5mm zone of inhibition was observed. The aloe vera gel with zero exposure to sunlight showed more effect towards the isolated microorganism with 1-5mm diameter zone of inhibition. Faecal thermo-tolerant isolated from oil polluted water showed resistance to all aloe vera gels exposed to sunlight. All aloe vera gels exposed to sunlight showed a high level disinfectant effect to faecal pathogenic *E. coli* isolated from oil polluted water with 3-8mm diameter zone of inhibition. Among the sunlight exposed aloe vera gel, those exposed for 10hrs showed more disinfectant of 2-8mm diameter zone of inhibition while those exposed for 4hrs had the least effect of 2-3mm zone of inhibition for six isolated microorganism.

The comparison of the efficacy of NaOCl and Aloe vera gel as disinfectants on the isolates showed that faecal thermo-tolerant coliform from the well water were inhibited at the highest zone of inhibition of 6mm by 5% NaOCl concentration, 5mm at 35°C and 12 mm at 10hr sunlight exposure of NaOCl. The Aloe vera highest disinfectant efficacy on the faecal thermo-tolerant coliform from the well water showed 5mm zone of inhibition at 5% concentration, 6mm at 0, 25 and 35°C and 5 mm at zero sunlight exposure time. Comparison of efficacy of NaOCl and Aloe vera gel as disinfectants on the isolates showed that faecal thermo-tolerant *E. coli* from the oil polluted water were inhibited at the highest zone of inhibition of 5mm by 5% NaOCl concentration, 5mm at 0 and 25°C and 4 mm at 10hr sunlight exposure of NaOCl. The Aloe vera highest disinfectant efficacy on the faecal thermo-tolerant *E. coli* from the oil polluted water showed 5 mm zone of inhibition at 5% concentration, 5 mm at 25°C and 4 mm at zero sunlight exposure time.

Furthermore, the results showed that faecal pathogen *E. coli* from the oil polluted water were inhibited at the highest zone of inhibition of 4 mm by 4% and 5% NaOCl concentration, 5 mm at 55°C and 4 mm at 8 and 10hrs sunlight exposure of NaOCl. The Aloe vera highest disinfectant efficacy on the faecal pathogenic *E. coli* from

the oil polluted water showed 1 mm zone of inhibition at all storage concentrations, 4 mm at 55°C and 8 mm zone of inhibition at 10hrs of sunlight exposure time. The faecal non-pathogen *E. coli* from the oil polluted water were inhibited at the highest zone of inhibition of 5 mm at 5% NaOCl concentration, 4 mm at 0 and 25°C storage temperature and 4 mm at 8 and 10hrs sunlight exposure of NaOCl. The aloe vera highest disinfectant efficacy on the faecal non-pathogenic *E. coli* from the oil polluted water showed 5 mm zone of inhibition at 5% storage concentration, 5 mm at 0 and 55°C storage temperature and 5 mm zone of inhibition at 10hrs of sunlight exposure time. The comparison of the efficacy of NaOCl and Aloe vera gel as disinfectants on the isolates showed that faecal thermo-tolerant coliform from the leachate sample were inhibited at the highest zone of inhibition of 5 mm by 5% NaOCl concentration, 3 mm at 35°C storage temperature and 4 mm at 8 and 10hrs sunlight exposure of NaOCl. The aloe vera highest disinfectant efficacy on the faecal thermo-tolerant coliform from the well water showed 7mm zone of inhibition at 3% concentration, 10mm at 0 and 25°C storage temperature and 5 mm at zero sunlight exposure time.

Efficacy of NaOCl and Aloe vera gel as disinfectants on the isolates showed that faecal thermo-tolerant *E. coli* from the leachate sample were inhibited at the highest zone of inhibition of 4mm by 4% NaOCl concentration, 5 mm at 55°C storage temperature and 5 mm zone of inhibition at 10hr sunlight exposure of NaOCl. The aloe vera highest disinfectant efficacy on the faecal thermo-tolerant *E. coli* from the leachate sample showed 8 mm zone of inhibition at 5% concentration, 9 mm at 0°C storage temperature and 3 mm at 6, 8 and 10hrs of sunlight exposure time. The isolates showed that faecal pathogen *E. coli* from the leachate sample were inhibited at the highest zone of inhibition of 1mm by all NaOCl concentration used in this study, 4 mm at 35°C of storage temperature and 3 mm at 10hrs sunlight exposure of NaOCl. The Aloe vera highest disinfectant efficacy on the faecal pathogenic *E. coli* from the leachate sample showed 3 mm zone of inhibition at 4 and 5% storage concentration, 3 mm at 0, 25 and 35°C storage temperature and 4 mm zone of inhibition at 10hrs of sunlight exposure time. The faecal non-pathogen *E. coli* from the leachate sample were inhibited at the highest zone of inhibition of 5 mm at 4 and 5% NaOCl concentrations, 5 mm at 0 and 25°C storage temperature and 5 mm at 0hrs sunlight exposure of NaOCl. The Aloe vera highest disinfectant efficacy on the faecal non-pathogenic *E. coli* from the leachate sample showed 3 mm zone of inhibition at 3% storage concentration, 5 mm at 0, 25 and 35°C storage temperature and 5 mm zone of inhibition at 10hrs of sunlight exposure time.

Regarding the statistical analysis of the efficacy difference between the aloe vera and NaOCl disinfectants, Minitab 17 paired t-test was used to determine the significant difference. For the well water, the only isolated microorganism was faecal thermo tolerant coliform (non-*E-coli*), and as given in Table 3, the p-values obtained for various values of storage concentration, storage temperature and sun exposure period of the aloe vera and NaOCl were 1,000, 0.468 and 0.247, respectively. All the p-values were greater than the criterion p-value which was chosen as α-level = 0.05. This indicates that there was no significant difference between the disinfection efficacies of aloe vera and NaOCl under the various experimental conditions investigated. In other words, aloe vera can be used as substitute for NaOCl without affecting the expected disinfection efficacy.

For oil polluted water, the three isolated microorganisms were faecal thermotolerant *E-coli*, faecal thermo tolerant non-*E-coli* and non-pathogenic faecal *E-coli.* As given in Table 4, the p-values obtained for various values of storage concentration, storage temperature and sun exposure period of the aloe vera and NaOCl for

disinfection against faecal thermotolerant *E-coli* were 0.778, 0.105 and 0.189, respectively. For disinfection against pathogenic faecal *E-coli*, the p-values were 0.011, 0.208 and 0.305 for various values of storage concentration, storage temperature and sun exposure period of the disinfectants, respectively. Regarding the disinfection against non-pathogenc faecal *E-coli*, the p-values were 0.749, 0.035 and 0.016 for various values of storage concentration, storage temperature and sun exposure period of the disinfectants, respectively. All the p-values for the disinfection of the isolated microorganisms from the oil polluted water were greater than the criterion p-values except the p-values for storage concentration values of disinfectants against pathogenic faecal *E-coli* (p = 0.011), storage temperature values of disinfectants against non-pathogenic faecal *E-coli* (p = 0.035) and exposure period to sunlight values of disinfectants against non-pathogenic faecal *E-coli* (p = 0.016)

For leachate, the four isolated microorganisms were faecal thermotolerant non- *E-coli*, faecal thermotolerant *E-coli*, non-pathogenic faecal *E-coli* and pathogenic faecal *E-coli*. As given in Table 5, the p-values obtained for various values of storage concentration, storage temperature and sun exposure period of the aloe vera and NaOCl for disinfection against faecal thermotolerant non- *E-coli* were 0.135, 0.006 and 1,000, respectively. For the disinfection against faecal thermotolerant *E-coli*, the p = values were 0.129, 0.305 and 0.208 for various values of storage concentration, storage temperature and sun exposure period of the disinfectants, respectively. Regarding the disinfection against non-pathogenic faecal *E-coli*, the p-values were 0.047, 0.242 and 0.646 for various values of storage concentration, storage temperature and sun exposure period of disinfectants, respectively. As regards the disinfection against pathogenic faecal *E-coli*, the p-values were 0.033, 0.587 and 0.016 for various values of storage concentration, storage temperature and sun exposure period of the disinfectants, respectively. As shown in Table 5, all the p-values for the disinfection of the isolated microorganisms from the leachate were greater than the criterion p-values except the p-values for storage temperature values of disinfectants against faecal thermotolerant non *E-coli*, (p =0.006), storage concentration values of disinfectants against non-pathogenic faecal *E-coli*, (p = 0.047) and pathogenic faecal *E-coli* (p = 0.033), as well as, exposure period to sunlight values of disinfectants against pathogenic faecal *E-coli*, (p = 0.016).

Generally, the p-values for using aloe vera and NaOCl as disinfectants under various conditions of concentration, temperature and sun exposure period ranged between 0.011 and 0.749 with most of the p-values indicating non-significant differences while few ones showed significant differences. On the average, the results showed that aloe vera could be used as a substitute for sodium hypochlorite for disinfection of water from multiple sources.

4. Conclusion

This study was set out to evaluate and compare the antimicrobial effects of sodium hypochlorite and Aloe vera gel, stored under various conditions and parameters, on coliform and *Escherichia coli* isolates from some water samples. The water samples collected were tap water, well water, oil polluted water and leachate All water samples were subjected to a presumptive test to evaluate the presence of coliforms in them and of which three of the water samples had a coliform values that were above the permissible count for drinking water by World health organization (WHO) and National Agency for Food and Drug Administration Commission (NAFDAC).

They were further subjected to a confirmatory test which showed that the well water contains faecal thermo-tolerant coliform, the oil polluted samples faecal thermo-tolerant *E. coli*, the leachate contains both faecal thermo-tolerant coliform and faecal thermo-tolerant *E. coli*. The further isolated faecal thermo-tolerant *E. coli* from the oil polluted water and leachate showed that both contained faecal pathogenic and non-pathogenic *E. coli*. The disinfectant efficacy of the NaOCl and Aloe vera was evaluated and compared on the eight isolated microorganisms from these three water samples using the disc diffusion method. The results of this study indicated that aloe vera and NaOCl were effective disinfectant against coliform isolated from polluted water source. The disifection efficacy of Aloe vera and NaOCl was more pronounced at higher concentrations and period of exposure to sunlight. Aloe vera, however seemed to be more stable in its disinfectant capability when compared with NaOCl at the storage temperatures especially at 0, 25 and 35^{o}C. Based on the results of this study, it could be concluded that Aloe Vera could be used as a substitute for sodium hypochlorite for disinfection of water from multiple sources.

5. Research Limitations (Constraints)

The scope of the study initially extended to the comparison of the antimicrobial effects of sodium hypo chlorite and Aloe vera gel; stored under various conditions including concentrations, pH, contact time between the disinfectants and microorganisms, temperature and sunlight exposure period, for the purpose of controlling microbial contamination of water samples. However, the results got from the study of the antimicrobial effects of sodium hypo chlorite and Aloe vera gel, stored under different conditions of pH and contact time between aloe vera gel / sodium hypochlorite disinfectants and microorganisms in the water medium were not included. The storage pH values (4.0, 5.0, 6.0 and 7.0) of aloe vera investigated were different from storage pH values (7.0, 8.0, 9.0 and 10.0) of sodium hypo chlorite. This was based on the premise that aloe vera was more effective at a lower pH while NaOCl at a higher pH. The results obtained could not however be testes using paired t- tests since different pH values were used for the aloe vera gel and sodium hypochlorite disinfectants. Similarly, it appeared that contact times of 0.5, 1.0, 1.5, 2.0 and 2.5 h between isolated microorganisms and the disinfectants including Aloe Vera gel and sodium hypochlorite in water medium were not long enough to induce a significant bacterial growth during the disc diffusion method to determine the antibiotic susceptibility of microorganisms to the disinfectants. Consequently, there were no observable effects of the Aloe vera gel and sodium hypochlorite disinfectants on the isolated microorganisms after the contact times of 0.5, 1.0, 1.5, 2.0 and 2.5 h, Del Carpio-Perochena and his colleagues [43] and Retamozo and his colleagues [49] reported that periods of time of 15, 20, and 30 min appeared to be insufficient for disinfectants to completely dissolve tissue and kill bacteria.

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