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EVALUATION OF BIOLOGICAL ANTIMICROBIAL EFFECT OF ALOE VERA ON COLIFORM ISOLATES IN LEACHATE FROM A DUMPSITE

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ABSTRACT

This study is to evaluate the biological disinfectant ability of Aloe vera (under different conditions and parameters) on coliform isolates from leachate that emanate from a dumpsite.Leachate samplewas subjected to a presumptive test to evaluate the presence of coliforms in them; the positive samples were further subjected to a confirmatory test to identify the thermo-tolerant nature of the coliform. The coliforms were thermo-tolerant *Escherichia coli* (FTE) and Thermo-tolerant coliform (non *E. coli*) (FTC) Disinfectant efficacies of Aloe vera (with various condition and parameters) were evaluated on the isolated microorganisms from the leachate samples, using the disc diffusion method. The Aloe vera showed that concentration of the disinfectant matters in its efficacy with 5% storage concentration showing high level of zone of inhibition with the isolated microorganisms. Also the Aloe vera was more effective at a lower pH and storage temperatures of 0, 25 and 35^{o}C.

Keywords: Leachate, Dumpsite, Aloe vera, Coliform, *Escherichia coli*

INTRODUCTION

Dumpsite wastes that are unmanaged had been associated with pollution of atmosphere, hydrosphere, and lithosphere part of the environment. This havoc subsequently generates some risk that could result in malicious outcomes (**Dermatas, 2017**).Wastes are subjected to different reaction and changes when disposed on landfills and some substances such as gases, elements and nutrients are set loose into the environment (**Koda *et al.*, 2013, Zornoza *et al.*,, 2016**). When these substances comes in contact with water on the dumpsite which could be from rainfall or any other sources through percolation, a leachate emanate (**Vaverková *et al.*, 2020**) as an effluent from the landfill to the surrounding.
Leachates toxicity can hardly be envisaged because of their specific constituents and synergic effects as pollutants (**Ancic *et al.*, 2020**).Leachates appear as a strongly coloured black, yellow or orange cloudy liquid when it emanate from typical dumpsite (**Agbozu *et al.*, 2015**). How long a dumpsite exists (its age) and types of waste it contains largely affect the composition and nature of a leachate. To evaluate the pollution potential of leachate from a landfill, its composition is essential and this could vary from chemicals, bacteria, fungi etc.(**De *et al.*, 2016**). **Michaela *et al.*., (2018**) reported a microbial population of total heterotrophic bacteria, total fungi, ranged from 6.23-9.79 Log cfu/ml, 5.25-6.64 Log cfu/ml respectively in leachate from a dumpsite in the Eastern part of Nigeria.
Aloe vera usage as drug can be traced back to 6000 years B.C (**Sharrif and Sandeep, 2011**). The plant like cactus does not needs much water for survival, and also saline soils does not hamper its survival (**Sharrif and Sandeep, 2011**). Aloe vera is a perennial plant that grows and take the shape of a tuft as shown in Figure 1. (**Spentzouris, 2015**)
Apart from water, anthraquinone glycoside along with barbaloin, C-glucoside, Aloesin, Aloesone and emodian are the predominant constituents of Aloe vera (**Deshpande, 2010**).in Aloe vera, Anthraquinones, which arephenolic compounds derived from quinonevaries from 4.5 to 30% (**Deshpande, 2010; Rodríguez *et al.*, 2013**). Additionally, in Aloe vera; Aloetic acid, emodincrygeminic acid, crysophenic acid, galactoronic acid, amylase, in conjunction with some steroids, organic acids, enzymes, aminoacids, saponins and minerals like calcium (4.7%), sodium (1.43%), potassium (6.6%), chloride (12.2%) and manganese (0.01%), are present (**Deshpande, 2010; Spentzouris, 2015**).
Aloe verahas been utilized for various applications including antimicrobial /antiseptic agents against fungi, bacteria and viruses **(Surjushe *et al.*, 2008**).
The aim of this study is to evaluate the biological disinfectant ability of Aloe vera under different conditions and parameters on coliform isolates from leachate from a dumpsite

Figure 1 *Aloe Vera* plant
Source: **Spentzouris (2015**)

MATERIALS AND METHODS

Preparation and Sterilization of Sample Bottles

The sample bottle was thoroughly washed and distilled water was used to rinse them. The steam sterilization of the bottles was done in an autoclave at 120ºC for 20 minutes. The bottle was allowed to cool before they were tightened using the stoppers and then stored in a refrigerator for its further use.

Collection of Sample

The method used by **Madrid and Zayas (2007**) with little modification was used in the sample collection. 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. The bottle cap/stopper, along with the aluminum foil, was carefully used to tighten the sample bottle without touching the neck of the bottle. The sample bottle was stored in melted ice/refrigerator and transported to the laboratory for analysis to be carried out within two hours. The leachate was called from Solous dumpsite at Igando in Alimosho local government area of Lagos, Nigeria with a geographical location of 6.56497N and 3.25131E

Preparation of Biological Disinfectant

The Aloe vera gel was extracted from a full grown plants, 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%). The 5% Aloe vera solution was further used to make Aloe vera

solution at varying pH of 4, 5, 6 and 7. Also a different storage temperature of 0, 25, 35, 45 and 55^{0}C was used to store 5% Aloe vera solution. Finally the 5% Aloe vera solution was exposed to sunlight for 0, 4, 6, 8 and 10 hours

Microbiological Analysis of Water

Microbiological analysis is a procedure which determines the concentration of bacteria and other microorganisms in water samples and from which inferences about the suitability of the water can be drawn. Most Probable Number (MPN) test was performed in three steps which include Presumptive test, Confirmatory test and Completed test to determine the count of isolates

Presumptive Test

Total coliform organisms in water sample was detected using presumptive test as a screening test. MacConkey broth containing Bromocresol purple was the culture medium used for the presumptive detection and isolation of coliform organisms in the water samples. Three rows of five tubes each were arranged in a test-tube rack. The tubes in the first row contained 10ml of double-strength (concentration) presumptive medium (MacConkey broth) while the tubes in the second and third rows contain 10ml of single-strength MacConkey broth.10ml of the100ml of water sample was added to each of the five tubes in the first row which contained double-strength presumptive medium.1ml of the100ml of water sample was added to each of the five tubes in the second row which contained single-strength presumptive medium. Finally, 0.1ml of sample was added to each of the five tubes in the third row which contained single-strength presumptive medium. The 15 test tubes were incubated at 35°C for 24-48 hours
At the end of the 48-hour incubation period, each test tube was gently shaken to observe acid formation and gas effervescence (streams of tiny bubbles) in the Durham tube. Acid formation was indicated by a yellow colouration of the broth. The tubes with gas effervescence and yellow colouration of the broth were considered positive. The number of positive tubes after 48 hours was recorded. Gas production and yellow colouration of the broth at the end of 24-48 hours' incubation were presumed to be due to the presence of coliforms in the sample.

Confirmatory Test

A confirmatory test was carried out in order to confirm and identify the presence of faecal thermo-tolerant coliforms which are *Escherichia coli* or non- *E.coli* that ferment lactose at 44–45º C. The carry out this test, two drops from each presumptive positive tube were transferred into five tubes containing 3ml of tryptone water. The five test tubes with the media were then incubated at (44–45ºC) for 24 hours. After incubation, 0.1mL of Kovacs reagent (paradimethyl amino bezaldehyde + Isoamyl alcohol + Sulphuric acid) was added into each of the five test tubes containing the media and the tryptone water. The test tubes were then mixed gently. There was an observation of the presence of indole indicated by a red colour in the Kovacs reagent, forming a film over the aqueous phase of the medium.
Confirmatory tests positive for indole, growth, and gas production showed the presence of thermo-tolerant *E. coli while* Growth and gas production in the absence of indole confirm thermotolerant coliforms. The number of positive tubes was recorded

Determination of MPN

The number of total coliforms was determined by counting the number of tubes giving positive reaction and comparing the pattern of positive results with standard statistical tables. The most probable number of (presumed) 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

Disinfectant activities of Aloe Vera on the isolated Microorganisms

Nutrient agar medium was used for growth inhibition study on the microorganisms using prepared Aloe vera subjected to different parameters. To examine the isolated microorganism susceptibility towards the biological antibiotic, disc diffusion method was used as the most practical method as illustrated by **Dohroo (2016**) with little modification due to the different conditions and parameters of the Aloe vera used. This antimicrobial susceptibility test was carried out using identified pathogenic microorganisms for investigation of the growth of microorganisms. The plates were incubated at 37±1ºC for 24h to see the effect of Aloe vera under various conditions (concentration, pH, storage temperature, exposure time to sunlight) on microbial growth and results were obtained.

RESULTS AND DISCUSSION

The leachate show very high values of coliform count >1800 CFU/ml from the result obtained in Table 1. Coliform bacteria can be opportunistic pathogens and are regarded as indicator organisms for fecal contamination (**Böger *et al.*, 2020**). The confirmatory test carried out in this study (Table 2) showed that the coliform present in the water sample were thermo-tolerant coliform that were both *Escherichia coli*, and non*E. Coli*

Table 1 Leachate and some physical parameters with the microbial counts

Samples	Color	Turbidity (NTU)	No of +ve tubes after 48 h 10ml-1ml-0.1ml	Coliform count (CFU/100 ml)	95% Confidence Limits	
					Lower	upper
Leachate	Dark brown	211	5-5-5	>1800	-	-`
WHO	Colorless	25		0 per 100ml		
NAFDAC	Colorless	5		10 per 100ml		

WHO- World Health Organization, NAFDAC- National Agency for Food and Drug administration and Control, +ve= Positive

The physical, chemical and microbiological water quality may have adverse effects on health and productivity (**Patience, 2012**).The occurrence of *E. coli* and coliforms,such as *Citrobacter*species (spp.), *Enterobacter*spp., *Klebsiella*spp. and *Proteus* spp. have been reported in some water sources (**Schulz *et al.*, 2014**).These fecal bacteria are a group of indicator bacteria that should not be present in drinking water supplied (National Research Council (**NRC), 2012**).

Figure 2 Presumptive test after incubation for 48 hours

Table 2. Leachate and class of coliform in them

Water samples	Confirmatory Test	
	Thermo-tolerant Coliform (non-*E. coli*)	Faecal Thermo-tolerant *E. coli*
Leachate	+	+

+= Presence

The Aloe vera gel has been reported to have a wide range of effectiveness against Gram positive and Gram negative bacteria (**Lawrence *et al*., 2009; Pareek *et al*., 2013**). Anthraquinones, phenols, saponins, terpenoids and enzymes present in Aloe vera gel had been associated with its antibacterial activity (**Lawrence *et al*., 2009; Pareek *et al*., 2013**).The antimicrobial agents of the plant were reported to effectively inhibit the growth, greatly reduce or kill several coliform bacteria such as *E. coli*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Klebsiella pneumonia* (**Gontijo *et al*., 2012; Irshad *et al*., 2011**; **Mariappan and Shanthi, 2012**). 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 leachate samples used as observed from the study.

Table 3 Aloe vera gel biological disinfectant effect in terms of diameter of clearance (millimeter mm) with disk diffusion method after 24hrs incubation at 37^{O}C

Conditions	Parameters	Leachate	
		FTC	FTE
Aloe Vera gel Storage Concentration (%)	1%	2	2
	2%	4	3
	3%	7	4
	4%	5	6
	5%	5	8
Aloe Vera gel Storage pH value	4.0	5	8
	5.0	4	13
	6.0	4	6
	7.0	3	9
Aloe Vera gel Storage Temperature (°C)	0	10	9
	25	10	5
	35	9	3
	45	6	3
	55	5	5
Sunlight exposure period of Aloe Vera gel (hr)	0	5	1
	4	2	2
	6	2	3
	8	2	3
	10	3	3

FTC: Faecal Thermo-tolerant Coliform (non-*E. coli*), FTE: Faecal Thermo-tolerant *E. coli;*

According to the result obtained from this study (Table 3), the 5% storage concentration displayed clearance of 5 and 8mm in diameterto both Faecal Thermo-tolerant Coliform (non-*E. coli*)(FTC) and Faecal Thermo-tolerant Coliform *E. coli* (FTE) respectively, the 1% displayed the least clearance of 2mm in diameter around the disc impregnated with the gel to inhibit the pre cultured of the FTC and FTE In the study by **Pareek *et al*., (2013)**, it was found that increase in strength of Aloe vera concentration resulted in reduction of total viable count of microorganisms.

In this study, from the different pH (4, 5, 6 and 7) of Aloe vera used, there was inhibition of growth of the coliform isolates, with pH values of 4 and 5 showing higher level of clearance ranging from 4-13mm of diameter. According to a study by **Tiwari. and Upadhayay, (2018)**, at different pH of 2, 4, 6 Aloe vera gel has shown to have a 100% recovery determinant at pH 4.The pH values of the formulated Aloe vera gels could play a role in supporting the carbopol in the gel-forming, it was reported in another study that pH values between 5 and 5.5 are sufficient to achieve a good viscosity and gel clarity (**Islam *et al.*, 2004**) since antiseptic gel effectivenessis related to its viscosity (**Kusuma *et al.*, 2019**)

In this study, at a storage temperature of 0 and 25^{o}C, the Aloe vera gel show more inhibition to the growth of the coliform isolates with5-10mm zone of inhibition. Also at a storage temperature of 35^{o}C, the Aloe vera gel was able to show its disinfectant efficacy by 9mm zone of inhibition to the FTC. In the study by **Kusuma *et al.*, (2019**) all forms 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 (**Ramachandra and Srinivasa, 2008**). But, the improper storage and length of time for storing herbal products could lead to degradation processes with consequent decrease in active substances resulting in metabolites with no activity, and the most extreme is the formation of toxic metabolites (**Thakur *et al*., 2011**).

The Aloe vera gel with zero exposure to sunlight was effective against the isolated microorganism with 1-5mm diameter zone of inhibition. The disinfectant capability of Aloe vera from the result in Table 3 decreases as the exposure to sunlight increases.

CONCLUSION

Aloe vera were effective disinfectants against coliform isolated from polluted water sources as it has been shown in this study and by **Pareek *et al* (2013**). Aloe vera showed that concentration of the disinfectant matters in its efficacy with 5% storage concentration showing high level of zone of inhibition with the isolated microorganisms. Aloe vera was more effective at a lower pH, storage temperatures especially at 0, 25 and 35^{o}C. Regarding the sunlight exposure period, Aloe vera effectiveness as disinfectants towards the coliforms isolates from the Leachate decrease as time to sunlight exposure increases.

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