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EVALUATION OF ANTIBACTERIAL EFFECT OF AN ENVIROMENTAL DISINFECTANT

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ABSTRACT
In chemistry, chemical compounds are molecular entities while in the healthcare sector; they are generally associated with disinfection and sterilization characteristics displayed in the environment. Disinfectant is an antibacterial agent that is applied on living and non-living surfaces, to destroy existing microorganisms on the skin and environment. It acts on microorganisms in either two ways: Growth inhibition or lethal action (bactericidal, fungicidal or virucidal effects), and was from compatible chemicals of analide grade (Chloroxylenol, phenolics, quaternary ammonium compound, hydrogen peroxide, ethanol and water), pĤ: 9.2, temperature: 20 - 60°C. Potency test result showed how some clinical isolates were kept under control, using Hugo and Russels agar well diffusion method. *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* were the assay organisms used, they displayed unequal zones of inhibition at a higher concentration (50% and 25%) while some of the assay organism showed no response at a lower concentration (12.5% and 6.25%), unlike *Bacillus subtilis* that is more responsive than the other assay organisms, referring the sample to be potent enough to inhibit some Gram positive strains and displayed some antibacterial effects though not as the standard. Other clinical analysis like MIC, MBC and MFC, have been recommended for further studies.

KEYWORDS: Antibacterial, Bactericidal, Environment, Disinfectant, Chloroxylenol

INTRODUCTION
Skin infections are self-timing and resolves on their own, but many others require medical attention. Microorganisms present on the skin are capable of rapid proliferation due to the presence of a nutritional source, high moisture content, suitable pH and temperature in the tanner (Lama, 2008). Bacterial skin infections technically have specific names based on their origin and extent, but even in the medical community, we often lump these technical terms into the common term “boil” (Wilkerson, 2002). Skin micro flora generally can be categorized into two groups: resident and transient flora. Resident florae establish secure attachments to the skin, present in stable numbers, and are able to tolerate an acidic environment, while transient florae are introduced from the environment and only attached, if the skin is disrupted. (Trent *et al*, 2002). In every environment, competition for food and space is one of the major factors that determine which organisms succeed and become established as the regions micro-flora (Falodun, 2006). Antibacterial agent, interferes with the growth and reproduction of bacteria and can prevent or kill the growth of micro-organisms like; heat, chemicals, UV light and so on. Ever since the identification of microorganisms as the causative agents of infectious diseases, various methods have been devised in reducing the population and prevalence of these organisms, like chemotherapy, immunization, sterilization and disinfection (Kim, 2007). These antimicrobial agents can be applied to non-living objects to destroy microorganisms, living on the objects, meaning they work

differently. Some disinfectants work in a way that will not only harm prokaryotic cells like bacteria, but they will harm eukaryotic cells like human. That is why some disinfectants can only be used on abiotic structures like tables, door knobs and so on, while others can be used on our skin, but not in our bodies. A few disinfectants can be used in our bodies in limited amounts (Miller *and Levine*, 2004), like antibiotics which destroy micro-organisms within the body and antiseptics, on living tissues. They destroy or irreversibly inactivate all test micro-organisms but not necessarily their spores. It destroys the cell wall of microbes or interfering with the metabolism. The mode of action of disinfectants differs greatly according to the chemical substance present and they can either be germicidal or micro-static in their effect. Any agent that oxidizes biological macro-molecules can be a good disinfectant; for example, most of the household cleaning agents such as ammonia and bleach (sodium hypochloride) are disinfectants. They can be sporo-static but are not necessarily sporicidal (Johnston *et al.,* 2002). Generally, *B. subtilis* and *S. aureus* are found to be the most sensitive bacteria being tested against disinfectants, while *P. aeruginosa* was the most resistant bacteria to these agents (Rasha, 2012). However, according to Chima (2011), proper disinfectant targets the reduction of the number of pathogens thereby reducing the frequency and intensity of disease occurrence. Disinfectant failure should be prevented as this could increase microbial load on fomites and predispose patients to certain microbial infections (Nwankwo, 2013). Disinfectants are classified in two broad categories: oxidizing and non-oxidizing disinfectants. Both categories are further subdivided into different categories. The most effective disinfectants are oxidizing agents, followed closely by reducing agents. Also bacteria may survive when contact time is suboptimal (Groom, 2003; Akabueze *et al*., 2013). Examples of agents are: Hydrogen peroxide (H_2O_2): (Russell & Russell, 1995). Hypochlorites (Chlorine): (Holleman and Wiberg, 2001). Iodine and Iodophors: (Okesola, 2011). Acids: (Maris, 1995). Alkaline solutions: (Jeffrey, 1995). Quaternary ammonium compounds: (QAC): (Quinn and Markey, 2001). The aim of this research, is to produce a disinfectant extract from an inorganic and organic chemical compounds, with the evaluation of its antibacterial effects**.**

MATERIALS AND METHODS

A clean bowl, turning stick, Phenol, Pine oil, Chloroxylenol, Isopropyl alcohol, Water, Hydrogen Peroxide, Formaldehyde, Colorant, Mueller Hinton Agar by biotech laboratories, Ethanol, McFarland turbidity standard 0.5**,** Water bath by Uniscope laboratories**,** Autoclave by Express Laboratories**,** Chilling Incubator (warm and cold temperatures) by Echoterm Laboratories**,** Lamina flow chamber by Esco**,** Cork borer size 10mm cross-section**,** Hot air oven by Uniscope Laboratories, Zone reader (ruler)**,** Chloroxylenol control standard for bacteria**,** saline tablet by Oxoid Laboratories**,** Pipettes (1ml,2ml,5ml,10ml,25ml). The adopted method by Russells and Hugo (1987) was confirmed on the potency test carried out on the research.

TEST PREPARATIONS

SAMPLE: The working samples were of four values: 50%, 25%, 12.5% and 6.25% solutions of the sample were achieved by mixing equal volume of water with the sample. Contents of bottles A, B, C and D were the working samples.

STANDARD: Similarly, working standards were of four values: 50%, 25%, 12.5% and 6.25% solutions of the standard were achieved by mixing equal volume of water with the samples. Contents of bottles A, B, C and D were the working standards.

MEDIA: The media were prepared following the inscription by the manufacturers. They were weighed and dispersed in the specified volume of distilled water. They were heated to melt in the water bath at $100^{\circ C}$. The molten agar gels so formed were dispensed in 25ml portions into

sample bottles and autoclaved at 121℃ for 15 minutes. The 25ml portion of the agar gel in each of the sample bottles was the working volume of the agar.

ASSAY ORGANISMS

The examination of the assay organisms was carried out by the use of the Agar well diffusion method. *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* (Gram negative), *Bacillus subtilis*, *Staphylococcus aureus* (Gram positive) are bacterial assay organisms used. These organisms, were primarily isolated on various diagnostic media and on Mueller Hinton Agar to remove toxins released from bacteria and it allows better diffusion than most other plates.

Calibration**:** The biological load of the assay organisms and bacterial suspensions were adjusted, using a sterile normal saline. The bacterial liquid cultures were added drop wise to the normal saline until the turbidity matched that of the densometer, 0.5 McFarland turbidity standards, then the suspension was used for the assay. Seeding**:** The assay medium which was prepared and measured was maintained at 45℃ so as to make it remain molten. 1ml of calibrated organisms was seeded into the warm agar and was mixed thoroughly using the roll-palm method before pour-plating. After solidifying under sterile condition in a biological safety cabinet, they were prepared for cork boring. Cork boring and dispensing of standards and samples: After allowing all the seeded agars to set, a cork borer, size 10mm cross-section was used for boring the wells. It was flamed and allowed to cool before using it to gently punch a hole in each of the sectors of the Petri dishes. All the cut portions were thrown into a dish of disinfectant. 0.15 ml of various working concentrations was dispensed into the wells and allowed to stand for four hours before incubation. Incubation of plates and readings: All the Petri dishes were incubated on lid-up position; it was so in order to avoid spillage,

after four hours on the Laboratory bench for the samples and standard concentration to diffuse the plates for antibacterial studies. They were incubated at 37℃ and observed after 24 hours as zones of inhibition was also observed due to bacteria growth. Reading of zones of inhibition**:** The inhibitory effects of the sample and control standards were exhibited by clear zones of no growth otherwise known as zones of inhibition. The measurement of these zones was carried out using the metric rule as the zone reader and the reading was done along the cross-section (diameter) of the zones so formed. The readings were in millimeters (mm) in accordance with the Clinical and Laboratory Standard Institute (CLSI), 2017.

STATISTICAL ANALYSIS

Data were expressed as mean of five replicates ± SD (standard deviation) the MIC, MBC and MFC. The values were subjected to student`s T – Test and data were considered statistically significant at $P< 0.05$.

RESULTS

Standards and samples analysis to determine the zones of inhibition of the assay organisms on the basis of different concentration.

Table1: Inhibition Zone Diameters (Mm) Of Standard on some bacteria

ASSAY ORGANISM	50%	25%	12.5%	6.25%
Escherichia coli	18.00 ± 01	14.00 ± 01	0.00 ± 04	0.00 ± 21
Salmonella typhi	18.00 ± 09	15.50 ± 15	12.00 ± 30	0.00 ± 10
Pseudomonas aeruginosa	18.00 ± 07	16.00 ± 32	0.00 ± 61	0.00 ± 06
Staphylococcus aureus	18.00 ± 03	13.50 ± 13	12.00 ± 04	0.00 ± 99
Bacillus subtilis	35.00 ± 08	22.00 ± 04	17.00 ± 31	13.00 ± 12

0.00 – Means no inhibition, while 12 mm and above means inhibition effect of that value

Table 2: Inhibition Zone Diameters (Mm) Of Sample on some bacteria

ASSAY ORGANISM	50%	25%	12.5%	6.25%
Escherichia coli	16.00 ± 02	12.00 ± 11	0.00 ± 08	0.00 ± 01
Salmonella typhi	15.00 ± 09	12.00 ± 09	0.00 ± 03	0.00 ± 04
Pseudomonas aeruginosa	15.00 ± 99	12.00 ± 03	0.00 ± 05	0.00 ± 03
Staphylococcus aureus	14.50	12.50	11.50	0.00
Bacillus subtilis	23.00	20.00	16.00	12.00

0.0 – Means no inhibition, while 12 mm and above means inhibition effect of that value

Graphical representation to compare the potency of individual bacterial.

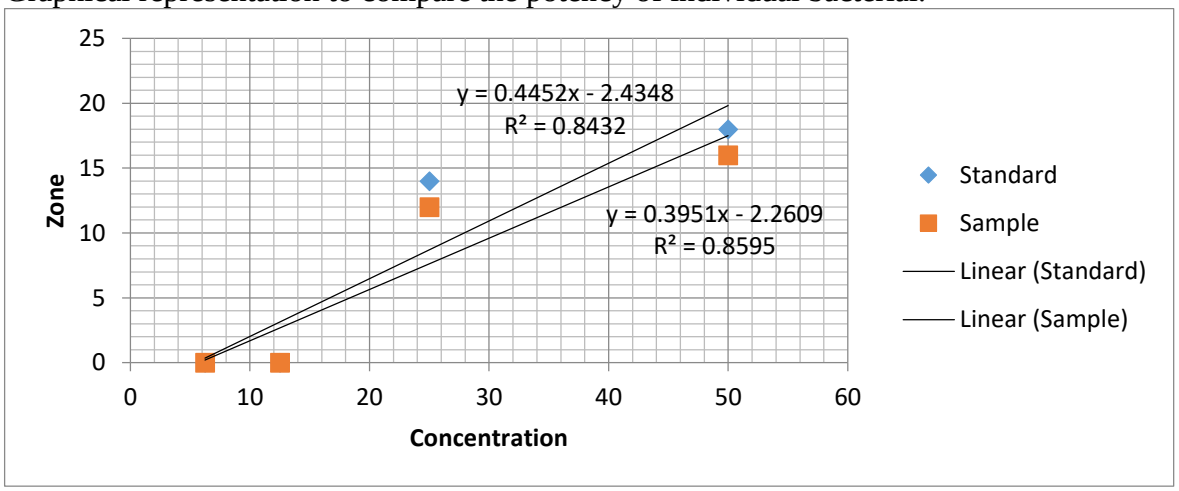

Figure 1: Inhibition for the standard and sample on *Escherichia coli*

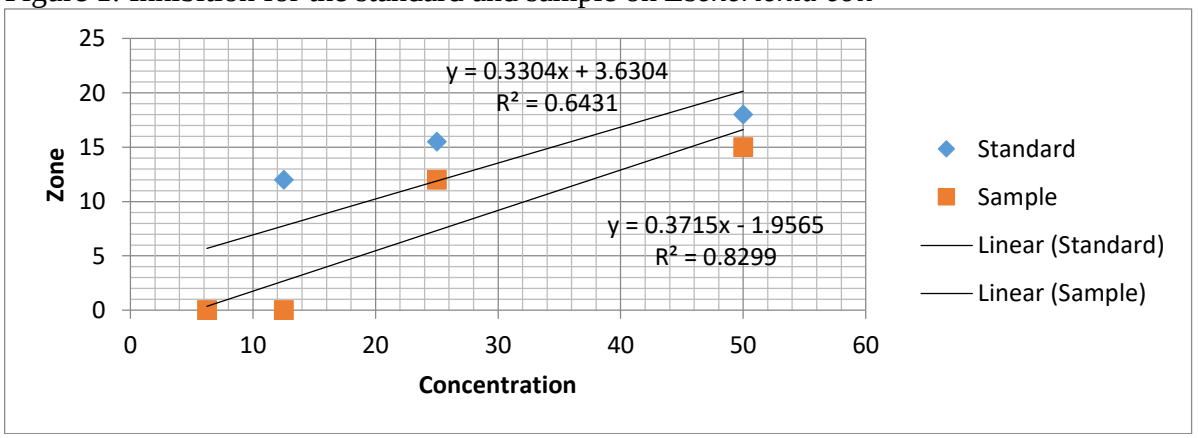

Figure 2: Inhibition for the standard and sample on *Salmonella typhi*

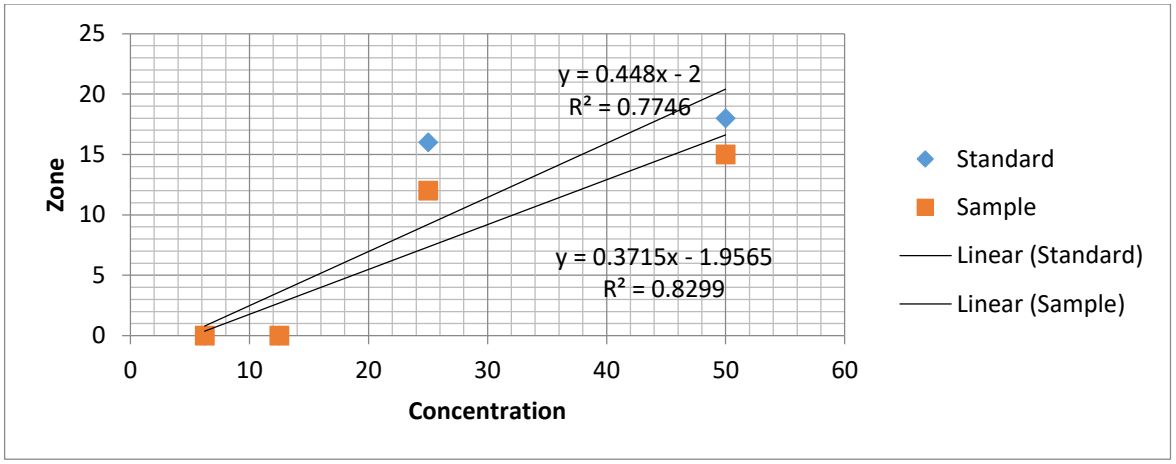

Figure 3: Inhibition for the standard and sample on *Pseudomonas aeruginosa*

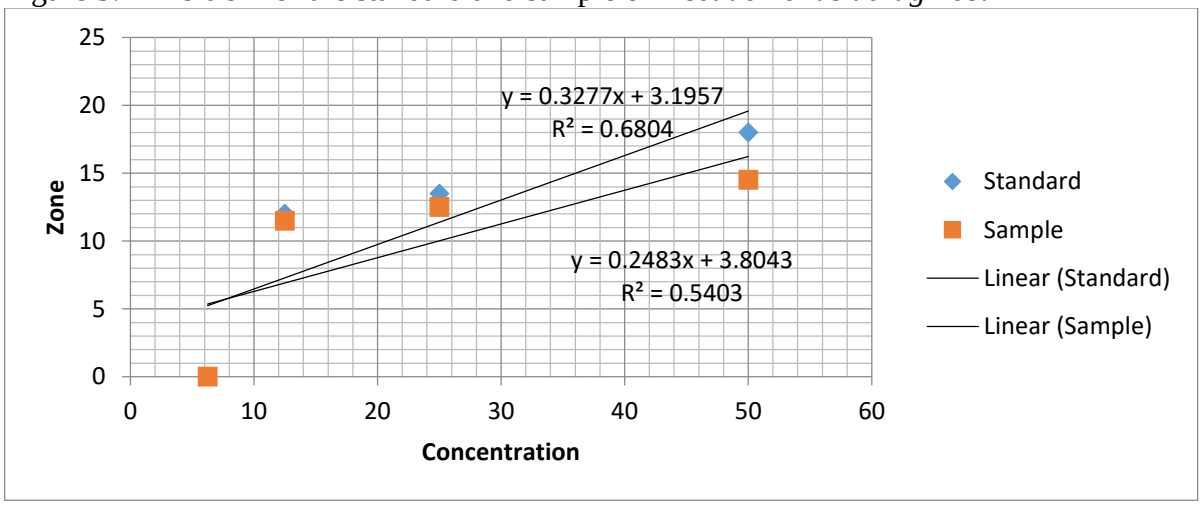

Figure 4: Inhibition for the standard and sample on *Staphylococcus aureus*

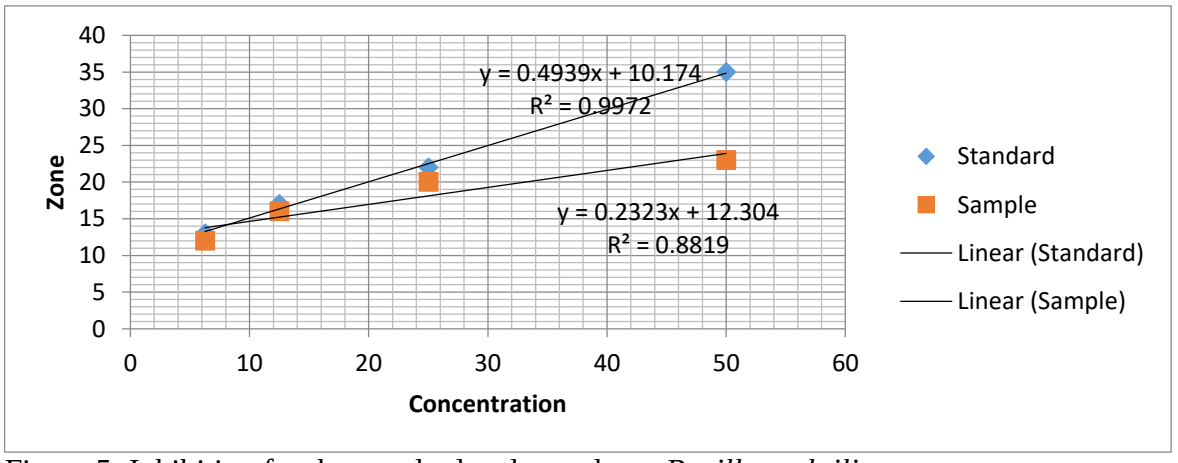

Figure 5: Inhibition for the standard and sample on *Bacillus subtilis*

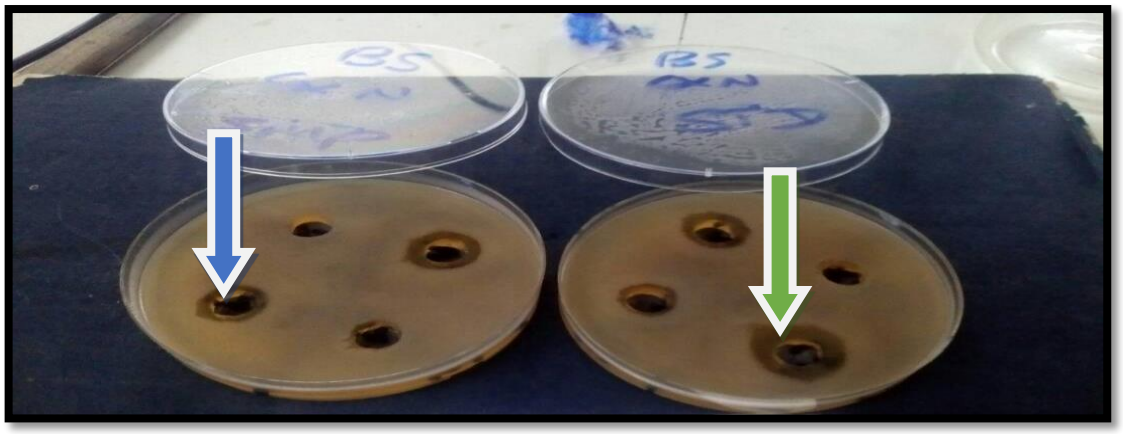

Fig. 6: The plates of *Bacillus subtilis* bacteria growth shows that the standard (brown arrow head) has a high zone of inhibition than the sample (Blue arrow head), when compared by observation.

DISCUSSION

Diseases caused by micro-organisms remain one of the major threats to human health. Although a number of antimicrobial agents have been isolated and developed to kill pathogenic micro-organisms effectively as reported by Barbe (2009), which is in agreement with the effective preparation of the sample of this study, used on five different Gram positive and negative bacteria which were known to be responsible for the prevalence of some common skin infections. The mean zones of growth inhibition of the isolates are a function of relative antibacterial activities of the sample and standard used. The larger zone of inhibition on some areas of the agar plate, which is free from microbial growth, exhibits the potency of the antibacterial (Larson, 1995), which agrees with the study, as it stipulates on Table1 & 2 also in figure 5 (*Bacillus S.)* above. Rasha, 2012 reported that *Pseudomonas aeruginosa* was the most resistant bacteria to disinfectants, while on the contrary, the bacterium was not resistant on the standard and sample (Table 1 & 2). It was observed that the potency of both the sample and the standard reduced with decrease in the concentration, as Table 1 (standard) showed more anti bacterial effect than the sample in Table 2 with decrease in concentration, which has a slight difference between each other in comparism as it agrees with Blancou, (1994) that the potency of an antibacterial sample decreases as the concentration reduces. It was also observed that some of the assay organisms showed no zone of inhibition at lower concentrations (in Table 1 & 2), *Escherichia coli, Salmonella typhi and Pseudomonas aeruginosa*, showed no response at the working values of 12.5% and 6.25% while *Staphylococcus aureus* was not responsive only at values equal to or less than 6.25%, which agrees with Akabueze *et al.,* (2013) that bacteria may also survive when contact time is sub-optimal, therefore disinfectant can be ineffective. The highest rates of inhibition were observed across all the bacteria examined at 50% concentration of both the sample and standard. It was also observed that the area of inhibition formed by *Bacillus Subtilis* was greater than other bacteria examined and even at low concentrations. Therefore, *Bacillus Subtilis* is more responsive than the other assay organisms, but there was slight difference between the standard and the sample which reduces the potency of the sample to an extent. However, this *in-vitro* disinfectant exhibited a higher rate of potency on *Bacillus subtilis* and its related diseases.

CONCLUSION

The antibacterial efficacy of this *in-vitro* disinfectant (from organic and inorganic compounds) was achieved, but it`s inhibitory effect at the various concentrations against the test organisms, revealed that it has slight antibacterial potential. Therefore other analysis can be recommended to establish a potent effect, for health, safety and environment.

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