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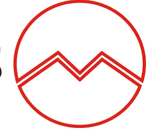

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THE ANTISEPTIC VALUES OF ROOT EXTRACT OF OKPOKPO (*Plumbago zeylanica)* IN DOMESTIC APPLICATIONS AMONG THE INHABITANTS OF IGBUZO COMMUNITY

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Abstract: The alcoholic extraction was done on the dried root of the plant using ethanol. The extract was used to determine its antibacterial activity on isolated bacteria and yeasts. The sensitivity of the different bacteria and the yeast isolates to the extract measured on agar gel diffusion test showed that Ecsherishia coli, Staphylococcus aureus, Streptococcus pyogenes, Klebsiella sp., Proteus mirabilis and Pseudomonas aureginosa recorded 3.4, 3.3, 3.6, 3.6, 3.0 and 2.9 mm while sensitivity of Pichia sp, Candida tropicalis and Sacharomyces cereviciae recorded 3.6, 3.3 and 3.8 mm respectively. The analysis of variance done on the various treatments showed that there was no significant difference in the activity of these treatments at $p<0.05$. The kinetics of the extract over a period of ten hours was time dependent recording a cell count reduction (log_{10}) of 1.12, 0.78, 0.84, 0.99, 0.37 and 1.2 for E.coli, S. aureus, Str pyogenes, K. pneumonia, P.mirabilis and Ps.aureginosa respectively. Heat has no significant effect on the activity of the extract. After 10 hours of treatment, the was a total cell count reduction from log_{10} 2.28 to log_{10} 0.36, 1.45, 1.24, 1.66, 1, 9 and 0.82 for E.coli, S. aureus, Str pyogenes, K. pneumonia, P.mirabilis and Ps.aureginosa respectively. The minimum inhibitory concentration (MIC), minimum bacteriocidal concentration (MBC) and minimum fungicidal concentration (MFC) demonstrated the high potency of the substance against bacterial and fungal species when compared with Dettol and Savlon. From the determination of the kinetics of the extract on tested microorganisms, a significant rate of microbial load reduction was established within 10 hours of treatment. It is safe and tolerated, as such can serve as an effective disinfectant in homes, offices and schools.
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INTRODUCTION

An increase in the emergence of multiple drug-resistant microbial species with the attendant health hazards associated with the use of chemical disinfectants is threatening the world population. Some chemical treatments in home such as the use of izal, dettol savlon and other forms of antiseptics are injurious to health hence the need for more friendly and tolerable substances. In the present scenario of antibiotic therapy and chemical treatments, there is a continuing quest for new antimicrobials from other sources including plants, since plants are known to produce diverse bioactive substances (Krishnaswami and Purushothoman 1980). The plant of interest in this study is *Plumbago zelanica* known among the people of Igbuzo as Okpokpo. It is a popular plant among Igbuzo people. This plant is of medicinal, pharmaceutical and therapeutic significances (Madhava Chetty, *et al.*, 2006; Agbaje and Adeniran, 2008; Dhale and Markandeya, 2011; Ravikumar and Sudha, 2011; Manu, *et al.,* 2012). Plumbagin found in *Plumbago* plant is an antimicrobial substance and have been used locally in the treatment of microbial infections in Igbuzo and its environs. Previous work done on the plant showed it has antibacterial activity against both gram-positive and gram-negative (Onianwah, unpublished). Onianwah (unpublished) and Thompson (1990) emphasized the use of leaves of *Plumbago zeylanica* in soup making and the root as chew stick. He added that alcoholic extract was used in the treatment of bacterial infection of reproductive and urinary tract. It is active against certain yeasts, fungi and protozoa (Mungwini, 2006; Ken, 2014). With the antimicrobial properties of the plant, it may be used in the treatment of water (Tyler: 2002; Verma; 2007) from various sources

that may be harbouring pathogenic microorganisms. Though, not much is known on the use of the plant as substitute for water treatment, development from other plants (Harikumar and Manjusha, 2013) proved cheaper and safer when compared to the conventional chemical treatments. For instance, Somani, *et al*., (2011) and Suarez *et al,* (2003) studied the effectiveness of natural herbs for antibacterial activity in water purification. The antimicrobial activity of Tulsi (*Ocimum sanctum*), Neem (*Azadirachta indica*), Wheatgrass (*Triticum aestivum*), Amla (*Phyllanthus emblica*) and Katakphala (*Strychnos potatorum*) were tested by Disc Diffusion Method (Kirby –Bauer Method) (Ranjan and Shivendu, 2006; Premanth *et al,* 2011;) after extracting the dried material powder of natural herbs in 50% alcohol (ethanol). An antibacterial activity was observed in all herbs used (Somani *et al*., 2011). In similar way, Harikumar and Manjusha (2013) assessed the antibacterial properties of certain selected herbs against different bacteria (Ernest and Pittler, 2000; Block, 2010) such as total coliforms, faecal coliforms, *Escherichia coli*, *Bacillus* sp. and *Serratia* sp. After the complete analysis of the antibacterial activity of different herbs, *Ocimum sanctum*, the most efficient herb, was selected and treatment methods based on the herb were developed so that it can be used conveniently in various household (Somani, 2006; Suarez *et al,* 2003).

P. zeylanica is a common plant around Igbuzo in Oshimili North Local Government Area of Delta State (Onianwah, unpublished). It is an herbaceous plant of immense cultural, nutritional and medicinal values within the local inhabitants. It is used, in addition to making cultural skin marks, also to treat ailments such as sexually transmitted diseases, food poisoning, stomach upset and dysentery. It has equally been used in oral washing and as chew stick. Very ancient practice involved its use as an agent of inducing abortion. The aim of this study is to explore the use of root extract of *Plumbago zeylanica* as sanitary agent for domestic and industrial uses. This will substitute the unregulated use of most chemical disinfectants in homes and industries. The survey study was done among the inhabitants of Igbuzo in Oshimili North Local Government Area of Delta State.

Plate 1: The plant *Plumbago zeylanica*

MATERIALS AND METHODS

Sample Area

Some domestic homes**,** schools and eateries in Igbuzo town in Oshimili North Local Government Area of Delta State were studied. Surfaces of tables and floors were swabbed using sterile clean cotton wools immersed in sterile distilled water. The used swabs were rinsed into sterile conical flask. The samples were immediately taken to the laboratory for microbiological analysis.

Extraction process

The roots of *Plumbago zeylanica* were obtained from a fallowed arable land in Igbuzo, Oshimili North Local Government Area of Delta State, Nigeria. The plant material was further identified by Prof. Grill of the Department of Botany, University of Benin, Benin City in Edo State, Nigeria. The roots were washed, dried and grinded into powder. 500 g of the powdered plant material was soaked in 70% ethanol for five days. Stirring was done every 24 hours using a sterile glass rod. After five days, the extract was passed through Whatman filter paper No. 1 (Whatman Limited, England). The alcoholic filtrate obtained was concentrated under vacuum at 30 °C and stored in a refrigerator for further use.

Materials used

Culture media used were MacConkey agar, Nutrient agar, Nutrient broth, Sabouraud agar and Biochemical media; and were prepared according to manufacturer's instruction (Oxoid, 2008). Poured plate method was used to enumerate the total microbial count (Okpokwasili, 2010; Onianwah, 2014).

Isolation of the test microorganisms

Aerobic bacterial and fungal species were isolated from home floors, funitures and sinks using basic microbiological techniques (Krieg and Holt, 1994; Onianwah, 2014). Streak and pour plate methods were used for the isolation and enumeration of the isolated microorganisms.

Biochemical tests were done to identify bacteria and yeasts species in line with Bergey's manual of determinative bacteriology (Krieg and Holt, 1994; Onianwah, 2014). Other fungal species were identified based on their cell and colonial morphology (Burnet and Hunter, 2006)

Determination of the antibacterial activity of the extracts

500 mg per ml of the extracts was screened for antibacterial activity using Well-in-Agar gel diffusion method of well size 3 mm in diameter (Cheesbrough, 2006; Onianwah and Stanley, 2016). Sensitivity of the tests microorganisms to the extracts against known antiseptic agents (Dettol and Savlon) was measured in millimeter (mm) after 18-24 hours aerobic incubation at 35 °C.

Total bacterial and fungal cell count:

A 1/10 serial dilution of the home floors, funitures and sink swabs (wash-up) was made in normal saline. 1 ml of each dilution seeded into each molten agar incorporated with 500 mg/ml of the extracts, mixed properly and poured into sterile petri dishes. The seeded media were allowed to solidify and then incubated at 35 °C for 18-24 hours. Growth as seen on different concentration plates were enumerated (Cheesbrough, 2006). This process is repeated after spraying the floors, furniture and sinks with 500 mg/ml concentration of the extract and enumeration done every hour for 8 hours.

Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimal Fungicidal Concentration (MFC)

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) of the extract was determined using the methods described by Cheesbrough (2006).

A 1 in 2 dilution of the stock extract (500 mg/ml) was made using physiological saline into 5 test tubes. A 0.5 ml of the test microorganisms inoculated into the various dilutions and incubated overnight at 35 °C. The overnight culture were inoculated into freshly prepared nutrient agar plate and incubated aerobically for 18-24 h at 35 °C. Fungal isolates were cultured into potato dextrose agar and incubated under the same conditions for measurement of minimal fungicidal concentration (Cheesbrough, 2006; Onianwah and Stanley, 2016; Onianwah *et al.*, 2020)

2.5 Kinetics of the extracts

0.1 ml of the test microorganisms was inoculated into a test tube containing 5 ml of Nutrient broth and 0.1 ml of the broth culture was taken from the test tube for bacterial cell count at time zero and subsequently, two hours interval for a maximum of ten hours. Enumeration of bacterial cells was done using the pour plate method (Krieg and Holt, 1994; Cheesbrough, 2006; Baker and Silverton, 1985; Okpokwasili, 2010; Onianwah and Stanley, 2016).

2.6 Effect of heat on extracts

The extracts were heated to boiling temperature. The precipitated white deposits were re-suspended in 1 ml of distilled water. Both the supernatant and the re-suspended deposits were tested for antibacterial activity using well-in-agar gel diffusion method (Cheesbrough, 2006; Baker and Silverton, 1985; Onianwah and Stanley, 2016).

2.7 Effect of relatively weak acid medium

The effect of pH was tested by adding to the extracts 0.1ml of 0.1N Hydrochloric acid to 5 ml of 500 mg extracts and the antibacterial activity of the compound tested using well-in-agar gel diffusion method (Cheesbrough, 2006; Onianwah and Stanley, 2016).

Results

Table 1: Biochemical reaction of bacterial isolates

Code	Grram	Urea	Citrate	Haemo;ysis	Indole	Oxidase	Glucose	Lactose	Galactose	Catalase	Coagulase	MR
A	+	-	-	-	+	-	A/G	+	A/G	-	-	+
B	+	-	-	-	-	-	A	+	-	+	+	-
C	+	-	-	α	-	-	A	+	-	-	-	-
D	-	-	+	-	-	-	A/G	+	A/G	-	-	+
E	-	+	-	-	-	-	A	-	-	-	-	-
F	-	-	-	-	-	+	A	-	-	-	-	-

From Table 1, *E.coli, S.aureus, S. pyogenes, Klebsiella sp, Proteus mirabilis* and *Ps. aureginosa* were isolated and characterized from samples collected from the different homes sampled. Similarly, fungal genera (Table 2) such as *Trichoderma, Aspergillus, Rhizopus,* and *Penicillium* were isolated and characterized. However, yeasts isolates were also found in the samples. Theses yeasts include *Pichia, Sacharomyces* and *Candida* as shown in Table 3.
The sensitivity of the different bacterial species to the extract measured on agar gel diffusion test showed that *E.coli, S. aureus, Str. pyogenes, Klebsiella sp., P. mirabilis* and *Ps.aureginosa* recorded 3.4, 3.3, 3.6, 3.6, 3.0 and 2.9 mm respectively, compared to the sensitivity of these bacterial species to Dettol (4.0, 3.8, 3.9, 3.9, 3.6 and 3.7 mm respectively) and Savlon (3.7, 3.9, 4.2, 3.3, 4.1 and 3.8 mm respectively). The analysis of variance done on the various treatments in Table 4 showed that there is no significant difference in the activity of these treatments at $p<0.05$. Similarly, the sensitivity of the yeast isolates (*Pichia sp, Candida tropicalis* and *Sacharomyces ceresviciae)* to the extract recorded as 3.6, 3.3 and 3.8 mm

Table 2: Colonial and Cell morphology of mould and Yeast isolates

Code	Colonial morphology	Cell morphology	Presumptive Organism
F1	Grey, rough edged and dry	Spherical, budding cells, single and occasional paired and	*Trichoderma* sp.
F2	Dry black, flat colonies with rough edge on PDA	Septate and branched mycelia. Conidia were in chains.	*Aspergillus* sp.
F3	Whitish fluffy colonies covering the entire plate brown/orange spores and whitish cotton like structures	Non septate hyphae, sporangiospores and black hemispherical columella	*Rhizopus* sp
F4	Whitish, convex, smooth and entire edged colonies	Spherical, sometimes oval and budding yeast cells in pairs or single	*Saccharomyces sp*
F5	A blue mold, velvet in shape with white edge	conidiophores was brush like in appearance which was single or paired and produces conidia. It had septate hyphae	*Penicillium sp*

The sensitivity of the different bacterial species to the extract measured on agar gel diffusion test showed that *E.coli, S. aureus, Str. pyogenes, Klebsiella sp., P. mirabilis* and *Ps.aureginosa* recorded 3.4, 3.3, 3.6, 3.6, 3.0 and 2.9 mm respectively compared to the sensitivity of these bacteria species to Dettol (4.0, 3.8, 3.9, 3.9, 3.6 and 3.7 mm respectively) and Savlon (3.7, 3.9, 4.2, 3.3, 4.1 and 3.8 mm respectively).

Table 3: Biochemical reactions of yeasts isolates

Code	Germ tube formation	Indole	Citrate	Glucose	Lactose	Mannitol	Sucrose	Isolate
H	+	+	+	-	-	-	-	*Pichia sp*
I	-	-	+	-	-	-	+	*Candida tropicalis*
J	-	-	-	+	-	-	-	*Sacharomyces sp*

The analysis of variance done on the various treatments in Table 4 showed that there is no significant difference in the activity of these treatments at $p<0.05$. Similarly, the sensitivity of the yeast isolates (*Pichia sp, Candida tropicalis* and *Sacharomyces cereviciae)* to the extract recorded as 3.6, 3.3 and 3.8 mm respectively as reported in Table 5.

Table 4: Agar gel diffusion test on bacterial isolates

Zone of inhibition in millimeter (mm), mean values.

Drug	*E.coli*	*S.aureus*	*Str.pyogens*	*Klebsiella sp*	*Proteus mirabilis*	*P. aureginosa*
Extract	3.4	3.3	3.6	3.6	3.0	2.9
Dettol	4.0	3.8	3.9	3.9	3.6	3.7
Savlon	3.7	3.9	4.2	3.3	4.1	3.8
Normal saline	0	0	0	0	0	0

Table 5: Agar gel diffusion test on yeast isolates (mean values)

	Zone of inhibition in millimeter (mm)		
Drug	*Pichia sp*	*C. tropicalis*	*Sacharomyces sp*
Savlon	4.2	4.0	3.9
Extract	3.6	3.3	3.8
Dettol	3.5	4.3	4.0
Normal saline	0	0	0

The analysis of variance done showed that there was no significance difference in the activity of the treatments at $p< 0.05$. Figure 1 illustrated the kinetics of the extract over a period of ten hours. From above, the activity of the extract was time dependent recording a cell count reduction (log_{10}) of 1.12, 0.78, 0.84, 0.99, 0.37 and 1.2 for *E.coli, S. aureus, Str pyogenes, K. pneumonia, P.mirabilis* and *Ps.aureginosa* respectively. Heat has no significant effect on the activity of the extract as shown in Figure 2. After 10 hours of treatment, the was a total cell count reduction from log_{10} 2.28 to log_{10} 0.36, 1.45, 1.24, 1.66, 1, 9 and 0.82 for *E.coli, S. aureus, Str pyogenes, K. pneumonia, P.mirabilis* and *Ps.aureginosa* respectively. Besides, the same pattern of activity was observed on the kinetics of the extract and effect of heat on the activity of the extract(Figures 3 and 4. The kinetics of the extract on yeast isolates read a total cell count of 0.54, 0.86 and 0.88 (log_{10}) while the effect of heat on the extract recorded a total cell count of 1.03, 0.73 and 0.8 (log_{10}). Table 5 showed the minimum inhibitory concentration and minimal fungicidal concentration of the extract on yeast isolates. Thus, at a minimum inhibitory concentration of 20 mg/l, *Pichia, Candida* and *Sacharomyces* reported a total cell count of 1.9, 2.06 and 0.56 to log_{10} while at the minimum fungicidal concentration of 40 mg/l, there was no cell growth.

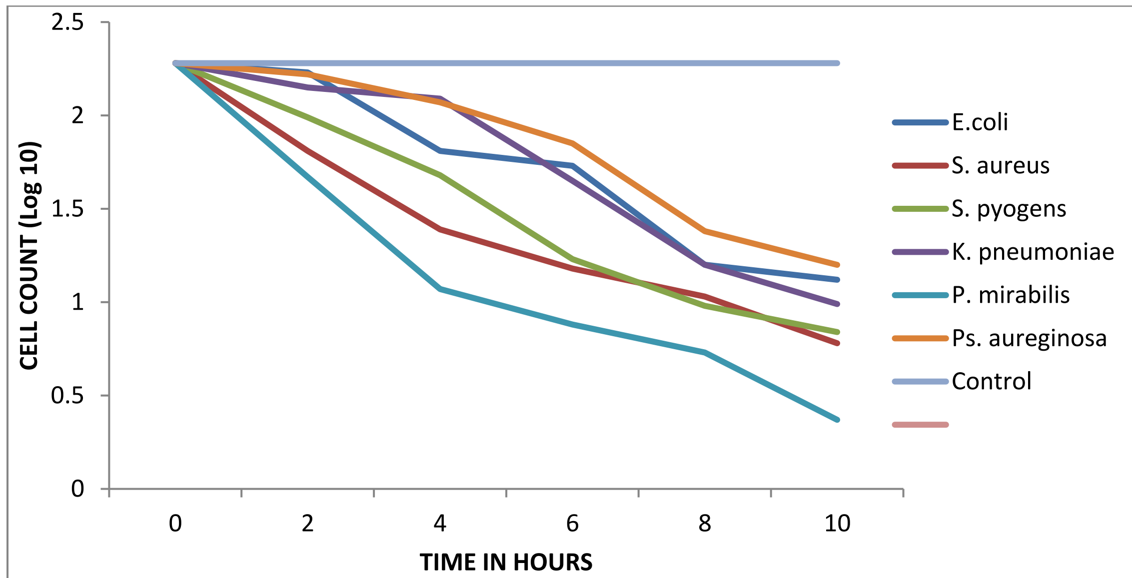

Figure 1: kinetics of extract on bacterial isolates

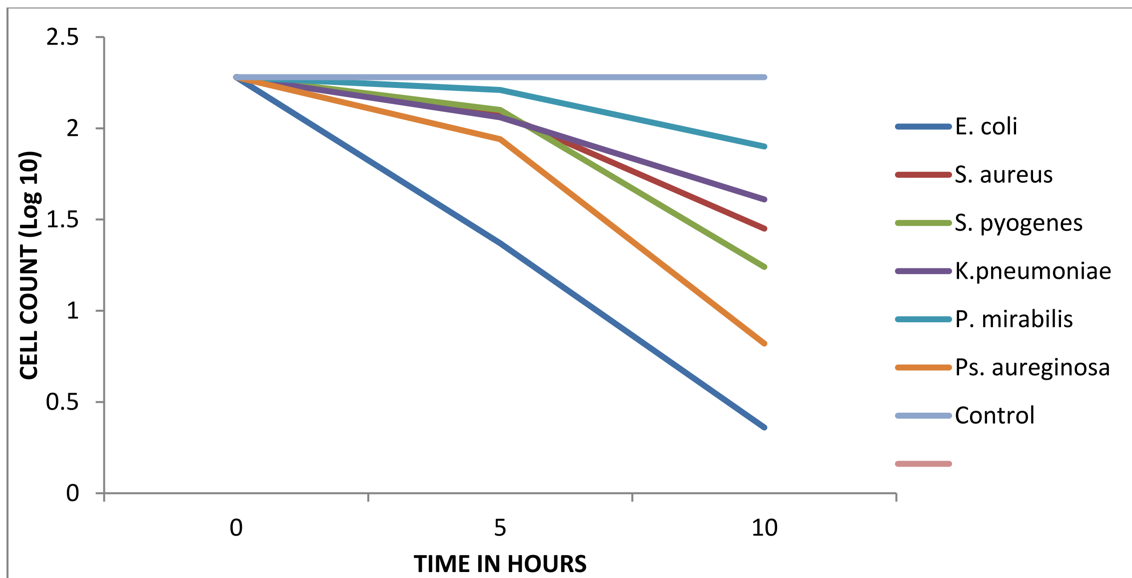

Figure 2: Effect of heated extract on bacterial isolates

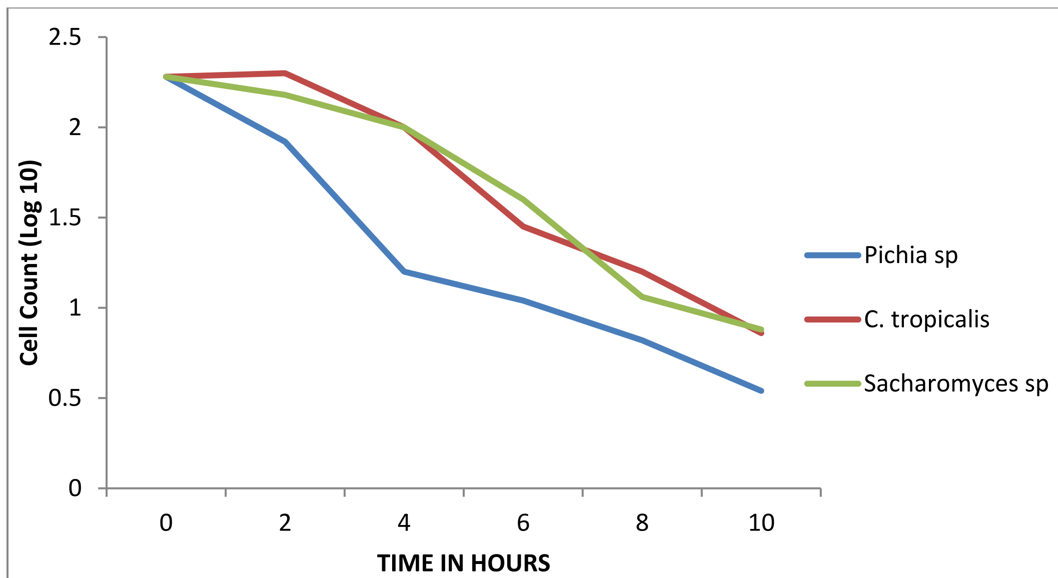

Figure 3: Kinetics of the extract on fungal isolates

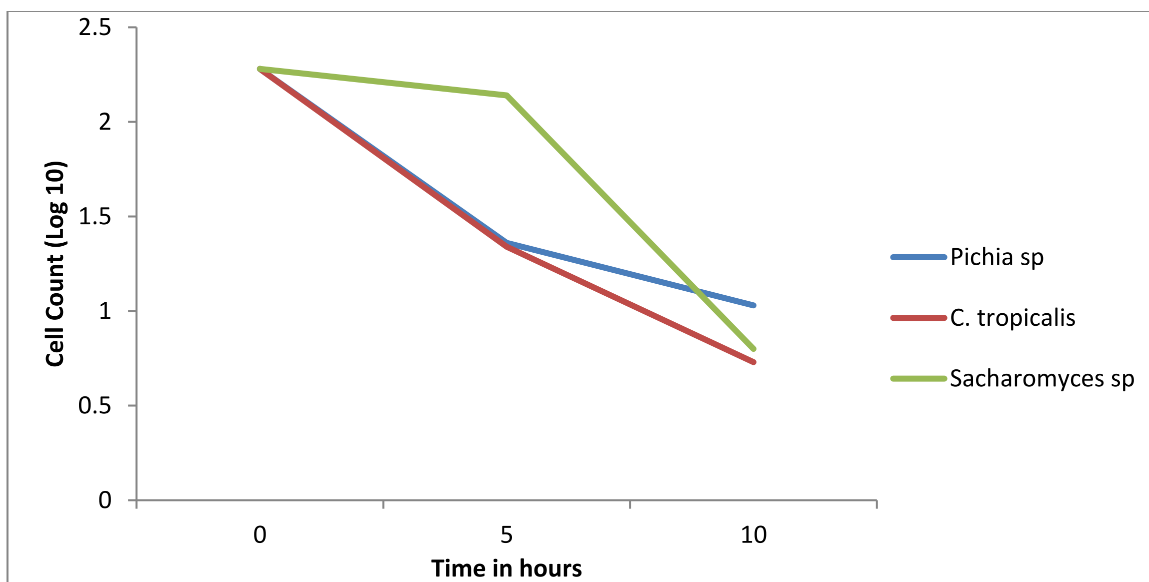

Figure 4: Effect of heated extract on fungal isolates

Tables 6a and 6b showed minimum inhibitory and minimal bactericidal concentration (MIC and MCB respectively) of the extract on the different bacterial isolates. While 10 mg/l was established for *Streptococcus pyogenes* and *Klebsiella pneumonia,* recording a total cell count of 1.18 and 2.09 to log_{10} ; 20 mg/l was the MIC for *E. coli* (1.9 log_{10}) and *Proteus mirabilis* (0.8 log_{10}), and 40 mg/l was the extracts MIC for *Staphylococcus aureus* (0.08 log_{10}) and *Pseudomonas aureginosa* (1.05 log_{10}). Also, the MBC for *Streptococcus pyogenes* and *Klebsiella pneumoniae* was 20 mg/l, the MCB for *Escherichia coli* and *Proteus mirabilis* was 40 mg/l and MCB for *Staphylococcus aureus* and *Pseudomonas aureginosa* was 80 mg/l.

Table 5: MIC/MFC of extract on fungal isolates

	C.tropicalis			*Sacharomyces sp*			*Pichia sp*			Control
Conc(mg/l)	Extract	Det	Sav	Extract	Det	Sav	Extract	Det	Sav	N. Saline
80	NG	NG	NG	NG	NG	NG	NG	NG	NG	2.28
40	NG	NG	NG	NG	NG	NG	NG	NG	0.68	2.28
20	1.9	1.24	0.94	2.06	NG	NG	0.56	0.8	2.03	2.28
10	2.09	2.5	1.67	2.28	1.28	2.05	2.04	1.9	2.28	2.28

Table 6a: MIC/MBC of extract on bacterial isolates

	E.coli			*P. aureginosa*			*S.pyogenes*			Control
Conc(mg/ml)	Extract	Det	Sav	Extract	Det	Sav	Extract	Det	Sav	N. Saline
80	NG	NG	NG	NG	NG	NG	NG	NG	NG	2.28
40	NG	NG	NG	1.05	NG	NG	NG	NG	NG	2.28
20	1.9	NG	0,8	1.93	0.65	NG	NG	NG	1.67	2.28
10	2..23	1.34	2.07	2.23	1.19	1.31	1.18	0.12	2.28	2.28

Table 6b: MIC/MBC of extract on bacterial isolates

	S.aureus			*K. pneumoniae*			*P.mirabilis*			Control
Conc(mg/ml)	Extract	Det	Sav	Extract	Det	Sav	Extract	Det	Sav	N. Saline
80	NG	NG	NG	NG	NG	NG	NG	NG	NG	2.28
40	0.08	NG	1.33	NG	NG	NG	NG	NG	NG	2.28
20	0.64	1.27	1.78	NG	NG	NG	0.8	NG	0.09	2.28
10	2.24	2.04	2.26	2.09	1.87	1.53	1.79	1.24	1.16	2.28

Acidifying the extract did not alter significantly the activity of the extract as shown if Figure 5.

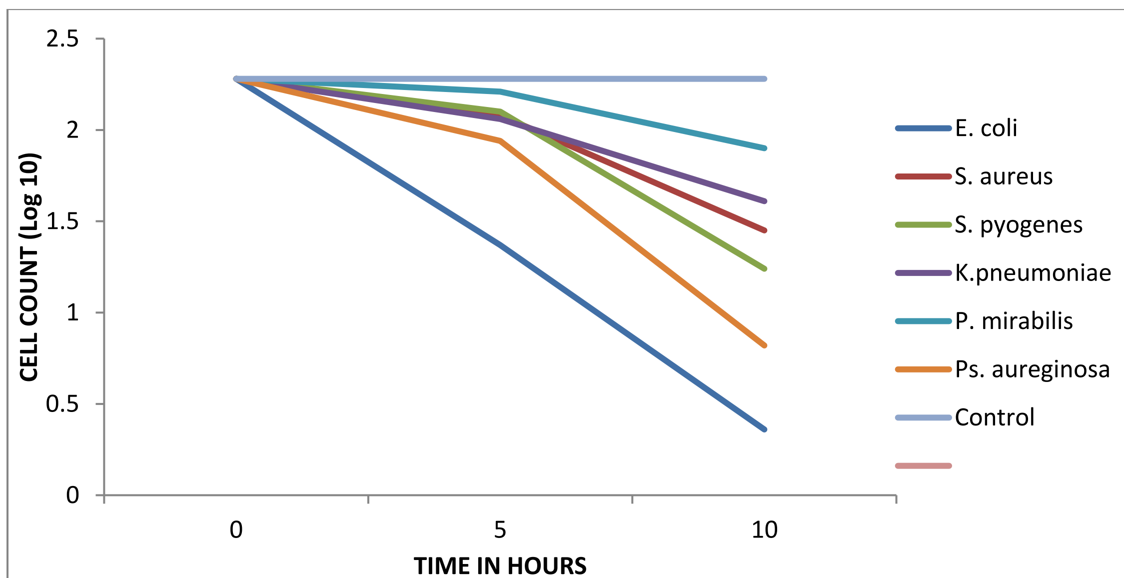

Figure 5: effect of weak acid on the activity of the extract

Discussion

Plumbago zeylanica has a bioactive substance (Krishnaswami and Purushothoman,1980; Ranjan and Shivenda, 2006; Premanth *et al,* 2011; Harikkumar and Manjusha, 2013) known as plumbagin that act against some gram positive and gram negative bacteria (Dhale and Markandeva, 2009; Ayoade *et al*., 2014) as shown in the sensitivity pattern in this study. According to previous research works done, the plant has demonstrated potential for medicinal, pharmaceutical and therapeutic significance (Madhava *et al.,* 2006; Agbaje and Adeniran, 2008; Dhale and Markandeya, 2011; Manu *et al.,*2012). This is evident from its pattern observed with some microorganis in this study. The extract demonstrated strong antimicrobial activity against *E. coli, Staphylococcus sp, Streptococcus sp, Klebsiella sp, Proteus sp* and *Pseudomonas sp.* This is in line with the work of Somani *et al.*(2006) and Onianwah and Stanley (2016) who observed antimicrobial activity of some herbs against total coliforms, feacal coliforms, *Bacillus sp* and *Serratia sp.*

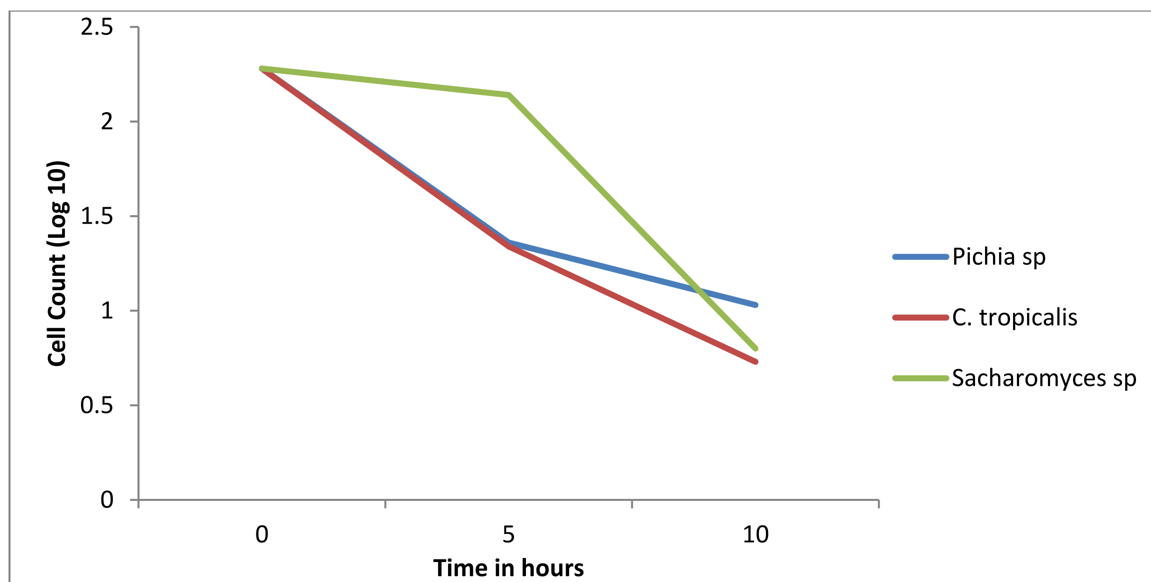

Figure 6: effect of weak acid on the activity of the extract

The extract was able to demonstrate antifungal activity against yeasts cells such as *Pichia sp, Candida sp* and *Saccharomyces sp.* This is supported by the works of Mangwini (2006) and Ken (2014) on the antibacterial and antifungal activity of *Plumbago zeylanica.* The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) recorded demonstrated high potency of the substance against bacterial and fungal species when compared with Dettol and Savlon as shown in this research work. As a consumable extract and pungent-less substance, it is acceptable, tolerated and very safe. Heat and relatively weak acid did not significantly affect the activity of the extract as evident from the research findings. This is supported by Onianwah (unpublished) and Manu, *et al.* (2012) opined that heating or addition of weak acid to the extract did not diminish its antimicrobial activity. From the determination of the kinetics of the extract on tested bacteria and fungi species, a significant rate of microbial load reduction was established within 10 hours of treatment. As such, it can serve as an effective disinfectant in homes, offices and schools. The ecologically friendly nature of the extract places it at advantage far above Dettol and Savlon and it is highly recommended for possible use.

Conclusion

The root extract of *Plumbago zeylanica* demonstrated potential as a bioactive substance to be used as a disinfectant in the treatment of bacterial and fungal contaminants. This is evident from its ability to eliminate most bacterial and fungal species found in most homes sampled. Its ability to record very high kinetic rate within a short time of ten (10) hours is quite commendable. From the results of this study, it is highly recommended that this extract be used in place of Dettol and Savlon to disinfect homes, schools, offices and other public facilities. It can also be used to clean floors, furniture, cabinets, kitchen utensils and plates, and can be applied in bath water to facilitate germs removal. It can, also, be used to disinfect equipment in the laboratories. The use of this extract is relatively cheap, easy to access in the study area and it is very safe..

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