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Full Length Research Paper

Antibiogram and the efficacy of leaf extract of *Chromolena odorota* (L.) R. M. King and H. Robinson on bacteria isolated from some swimming pools within Akure metropolis

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Microorganisms being cosmopolitan are widely distributed in nature and can as well be found in swimming pools. These microorganisms contaminate swimming pools and other recreational water through indiscriminate defecation, contamination from rodents and birds etc. making these sources of water threat to human life. Concurrently, the resistant to conventional antibiotics has also increased the danger on people that are exposed to swimming pools, thus there is the need for alternative therapy. In view of this, study revealed the antibiogram of microorganisms isolated from swimming pools within Akure metropolis and the efficacy of *Chromolena odorata* as an alternative therapy to conventional antibiotics. The isolation, identification and antibiotic sensitivity of bacteria from selected swimming pools in Akure was carried out. Ethanol extract of the leaf of *C. odorata* was further tested on the isolates for comparative purpose with the commercial antibiotics using standard methods. The results of the experiment showed that the highest bacterial load of 7.10×10^3 cfu/ml was obtained for Swan hotel, while the least bacterial load was recorded for Sun view hotel with a bacterial load of 2.83×10^3 cfu/ml. Bacteria that were isolated and identified are *Staphylococcus aureus, Proteus vulgaris, Staphylococcus epidermidis, Bacillus subtilis* and *Pseudomonas aeruginosa.* The antibiotic sensitivity of the isolates showed that ofloxacin and ciprofloxacin were able to exert inhibitory effect on all the isolates. Most of the antibiotics were however resisted by *P. aeruginosa*, except ofloxacin. The inhibitory evaluation of ethanol extract of *C. odorata* on the bacterial isolates showed that the extract had its highest inhibitory effect on *Proteus vulgaris* with a zone diameter of 7.9 mm. The extract was however resisted by *P. aeruginosa*. These results showed that these swimming pools house a variety of microorganisms, some of which are pathogenic and should therefore be disinfected on regular basis to prevent dissemination of these bacteria by swimmers. *C. odorata* is also is a good alternative antimicrobial agent especially to *P. vulgaris*.

Key words: *Chromolena odorata, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris,* swimming pools.

INTRODUCTION

"Public Pool" means an artificial basin constructed of concrete, steel, fiberglass or other relatively impervious material intended for recreational bathing, swimming, diving, or therapeutic purposes which is located either indoors or outdoors and is provided with a controlled water supply and which is not used or intended to be used as a pool at a single family residence (GSRWE, 2000). The term also includes a pool located at a single family residence which is used or intended to be used for commercial or business purposes.

Microorganisms can be found in swimming pools and some other recreational water environments. These microorganisms may be introduced in a number of ways. Basically, the risk of infection has been implicated to feacal contamination of the water which may be as a result indiscriminate release of feaces by bathers, through contaminated source water or animals such as birds and rodents (CDC, 2001). Feacal matter can also be introduced into the water when a person has an accidental feacal release – AFR (through the release of formed stool or diarrhoea into the water) or residual feacal material on swimmers' bodies is washed into the pool (CDC, 2001). Proper management of pools would have to the large extent prevented or reduced outbreaks related to swimming pools. Whereas, non feacal contaminations can be linked to the shedding of vomit, mucus, saliva or skin into the swimming pools by humans. These can be a major source of infection in other users of the pools (CDC, 2001). The release of these pathogens (opportunistic pathogens, viruses and fungi) can result to the development of skin infections, diarrhoea, among others. Similarly, public swimming pools may be exposed to pathogens from pests and rodents which can habour lassa fever virus, ebola virus etc. especially when the environment is not properly cleaned (GSRWE, 2000).

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value (Nostro et al., 2000). According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Ammara et al., 2009). *Chromolaena odorata* (L. *f.*) King and Robinson (synonym: *Eupatorium odoratum* L.) (Asteraceae) is a perennial scandent or semi-woody shrub. In traditional medicine, a decoction of the leaf is used as a cough remedy and as an ingredient with lemon grass and guava leaves for the treatment of malaria (Iwu et al., 1999). Other medicinal uses include anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory and diuretic (Iwu et al., 1999). A decoction of flowers is used as tonic, antipyretic and heart tonic (Bunyapraphatsara and Chokechaijaroenporn, 2000).

The significance of the study is to show the various microorganisms of medical importance that are associated with swimming pool and to investigate the effect of *C. odorata* on the microorganisms isolated as an alternative treatment to antibiotics.

METHODOLOGY

Collection of samples

Sterile bottles (20 ml Plastilab containers made by Agary Phamaceuticals in China) were used to collect swimming water samples (20 ml each from 6 different points-surface, bottom and mid-debt of up and down sides of each swimming pool) from selected hotels within Akure metropolis namely; Sunview Hotel located at Alagbaka. First Victoria, Bliss World at Ijapo Estate and Swan located along Ilesha-Akure Express way. These are the four major hotels in Akure metropolis with standard public swimming pools. Also, these hotels are strategically located in the four cardinal points of Akure (East, West, North and South of the town; hence their choice). In addition, these pools are mostly crowded on daily basis which makes them preferred for this research. The collection was done in triplicates and transferred in an ice packed container within 1 h of collection to the Department of Microbiology laboratory, Federal University of Technology, Akure (FUTA) for necessary analyses.

C. odorata a common plant in Southwestern Nigeria was collected from bushes around Southgate area of The Federal University of Technology, Akure. The plant was taking to the Department of Crop Soil and Pest Management, Federal University of Technology, Akure for authentication. The plant was further identified as *C. odorata* by Prof. Oyelana, O. A.; a renown botanist in Elizade University, Ilara-Mokin, Ondo State, Nigeria.

Isolation of microorganisms

The four samples each from the study swimming pools were cultured on already prepared nutrient agar in triplicates, using the pour plate and streak method. Distinct colonies of bacteria were picked using sterile inoculating loop. These were streaked onto the surface of the prepared nutrient agar plate to obtain pure isolates for confirmation of their identities. Gram staining was carried out on the subculture to ascertain purity. Pure isolates were sub cultured on a double strength nutrient agar slant for further studies and identification. Cultural characterization of colonies; color, edge, elevation, surface, biochemical tests such as catalase, oxidase, indole production, coagulase, methyl red and citrate test as well as sugar fermentation (such as glucose, arabinose, fructose, maltose, sucrose, lactose, galactose, etc) using conventional methods (Holt et al., 1994) were employed.

Preparation of the leaves

The plant materials were collected in the afternoon of a sunny day in the rainy season and washed to reduce microbial load to a large extent. They were further air dried to remove water on the leave surfaces. The leaves were further dried in the laboratory oven at 60°C for two days. After sufficiently dried, a warring industrial blender was used to crush the leaves to powder and then weighed.

Ethanolic extract preparation

A hundred gram of pulverized powdered leaves of plant materials

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were weighed using an electronic weighing balance and weighed sample were soaked separately in a clean 250 ml conical flasks containing 200 mls of 98% ethanol. The mixture was vigorously stirred with a stirrer. After 72 h with interval stirring, the mixture was filtered using a clean filter paper (Whatman filter paper) into a clean beaker and the filtrate was concentrated to dryness by evaporation using a steam bath at 90°C for 48 h. The filtrates were concentrated by evaporation using rotary evaporator. The standard extracts obtained were then stored in the refrigerator at 4°C as stated by (Mbajiuka et al., 2014).

Antibiotic sensitivity test

The antibiotic sensitivity test was carried out in order to know the sensitivity of the microorganism to the different commercially available antibiotics. These antibiotics discs include: Augmentin, Amoxacillin, Ofloxacin, Gentamycin, Cotrimoxazole, Nitrofuranton, Nalidixic acid and Tetracyclin. Disc diffusion method was used to determine the effect of standard antibiotics on the bacterial isolates as described by Jayasingh and Parkinson (2008). Sterile Petri dishes were seeded aseptically with 1 ml each of 18 h old pure cultures of the test organisms each while about 15 ml of sterilized Muller-Hinton agar was poured aseptically on the seeded plates. The culture was first standardized using spectrophotometer and plate count methods at 2.0 $\times10^4$ cfu/ml. McFarland standard at 540 nm (0.050 spectrophotic reading) was used. The plate were swirled carefully for even distribution and allowed to gel. With the aid of sterile forceps the antibiotics discs (Optu standard antibiotic discs made by Optu medicals and equipments, United Kingdom) were placed firmly on solidified plates and incubated for 24 h at 37°C. After incubation, clear areas around the disc represent the zones of inhibition and the areas without clear zones were also observed. Seeded agar plates without antibiotics disc served as the control experiment. The zones of inhibition were measured in millimeter (mm). The experiment was carried out in triplicate.

Susceptibility of isolates to extracts

Mueller Hinton agar plates were inoculated with respective test organisms using syringe and needle. This was then streaked for each test organism. Plates were in triplicate for each test organism for the extract. The plates were allowed to set properly for 15 min in a lamina flow. Using sterile cork-borer of 4 mm diameter well was made on the streaked plate of Mueller Hinton agar with the test organisms. About 0.4 ml of 100 mg/ml concentration of the ethanol extract of *C. odorata* was introduced into the well. This was incubated at 37°C for 18-24 h to observe the zone of growth inhibition produced by the extract (Mbajiuka et al., 2014).

Statistical analysis of result

Results obtained were subjected to descriptive one way analyses of variance, SPSS version 16 Microsoft windows 7 and Duncan multiple range tests was used as follow up test.

RESULTS AND DISCUSSION

Table 1 shows the mean microbial load of from the four swimming pool water assayed for. The sun view hotel had the least microbial load of 2.83×10^3 cfu/ml, while swan hotel had the highest microbial load of 7.10×10^3 cfu/ml. Figure 1 on the other hand showed the percentage

Table 1. Mean microbial load of swimming pools water samples.

S/N	Sample source	Mean microbial load (cfu/ml)
1	Sun view hotel	2.83×10^3
2	Bliss world hotel	3.30×10^3
3	First Victoria hotel	5.90×10^3
4	Swan hotel	7.10×10^3

occurrence of the different bacteria isolated from the swimming pool water of the different hotels. The most prominent of the bacteria isolated which was present in all the four hotels swimming water is the genus *Staphylococcus,* especially *S. aureus. Proteus vulgaris* was isolated from Bliss world hotel swimming water, while *Pseudomonas aeruginosa* was isolated from First Victoria and Swan hotels respectively.

Figure 2 shows the diameter of zones of inhibition of commercial antibiotics on the bacterial isolates. *Bacillus subtilis* was the most susceptible to all the commercial antibiotics while *P. aeruginosa* was the least susceptible to all the commercial antibiotics used. Table 2 shows the diameter of zones of inhibition of ethanol extract *C. odorata* on the bacterial isolates. *P. vulgaris* was the most susceptible bacteria with a diameter of zone of inhibition of 7.90±1.05 mm while *P. aeruginosa* was completely resistant to the extract.

Table 3 and Figure 3 shows minimum inhibitory concentration of ethanol extract of *C. odorata* on the bacterial isolates as well as the diagrammatic comparative diameter of zones of inhibition of commercial antibiotics and ethanol extract of *C. odorata* on bacterial isolates.

DISCUSSION

The results obtained in this work have shown that most swimming pool habour different species of bacteria. According to Yoder et al. (2004), the swimming pool cannot be sterile as it is often found in an open space or air area. However, according to World Health Organization procedure for owning and operating a swimming pool, a strict adherence to the constant sterilization with the use of chemicals such as chlorine to sterilize the water in order to minimize contamination must be followed (WHO, 2008). Although, no special regulation for the microbial level or load for swimming pool water, the microorganisms as well as the microbial level should not be too high. Aho and Hirn (2001), already reported that the higher the microbial load, the greater the risks of the presence pathogenic bacteria.

The presence of bacteria such as *S. aureus, P. vulgaris, B. subtilis* and *P. aeruginosa* isolated from these swimming pools poses questions such as how harmful are they, how did they get into the water and can people

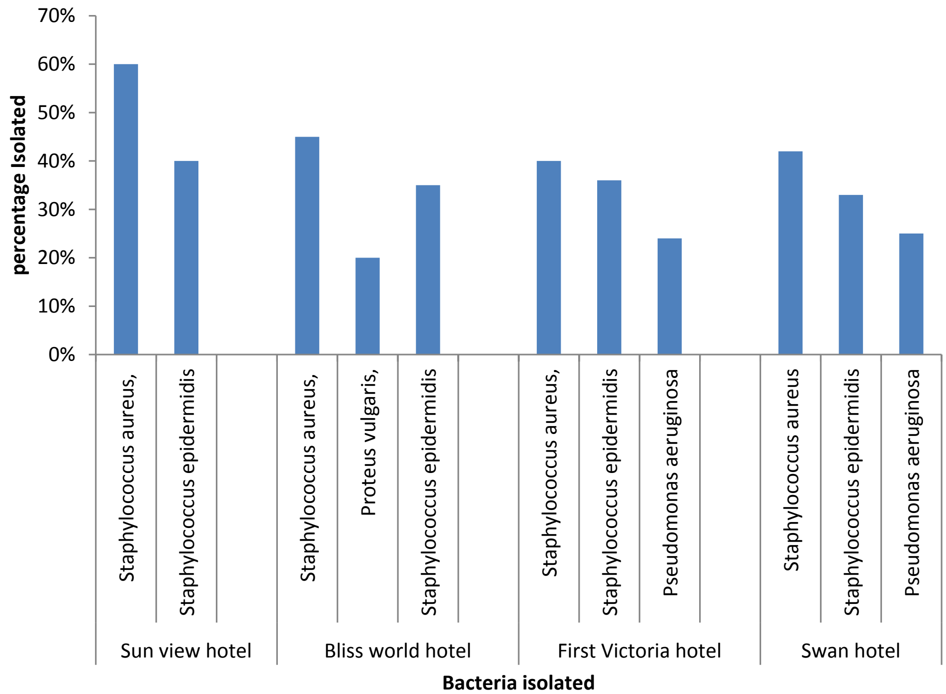

Figure 1. Percentage occurrence of bacteria isolated.

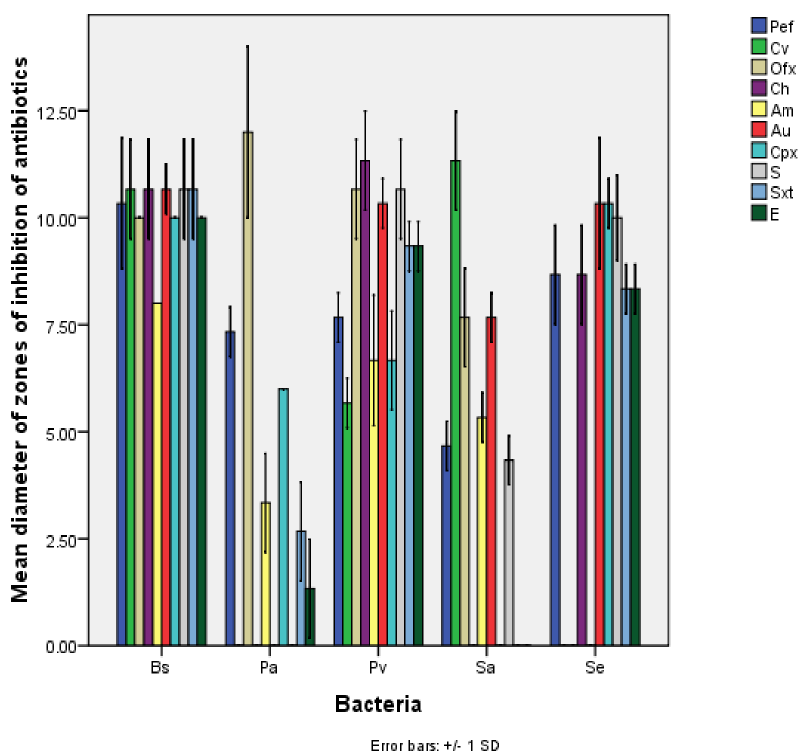

Figure 2. Diameter of zones of inhibition of commercial antibiotics on bacterial isolates. Legend: Bs-*Bacillus subtilis*, Pa- *Pseudomonas aeruginosa.* Pv- *Proteus vulgaris,* Sa- *Staphylococcus aureus,* Se- *Staphylococcus epidermidis*; Pef- Pefloxacin, cv- Gentamicin; ofx- ofloxacin, Au- Augumentin, Cpx- Ciprofloxacin, S- Streptomycin, Sxt- Septrin, E- Erythromycin, Am- Ampicillin, Ch- Chloramphenicol.

Table 2. Diameter of zones of inhibition of ethanol extract of *Chromolena odorata.*

Isolate number	Bacterial name	Diameter zones of inhibition (mm)
1	*Staphylococcus aureus*	5.45±0.50[b]
2	*Proteus vulgaris*	7.90±1.05[c]
3	*Staphylococcus epidermidis*	5.20±0.10[b]
4	*Bacillus subtilis*	2.00±0.00[a]
5	*Pseudomonas aeruginosa*	0.00±0.00[a]

Values in the same row carrying the same superscript are not significantly different according to Duncan's multiple range tests at (P≤0.05).

Table 3. Minimum inhibitory concentration of ethanol extract of *Chromolena odorata.*

Isolate number	Bacterial name	Minimum inhibitory concentration (mg/ml)
1	*Staphylococcus aureus*	50
2	*Proteus vulgaris*	100
3	*Staphylococcus epidermidis*	50
4	*Bacillus subtilis*	200
5	*Pseudomonas aeruginosa*	200

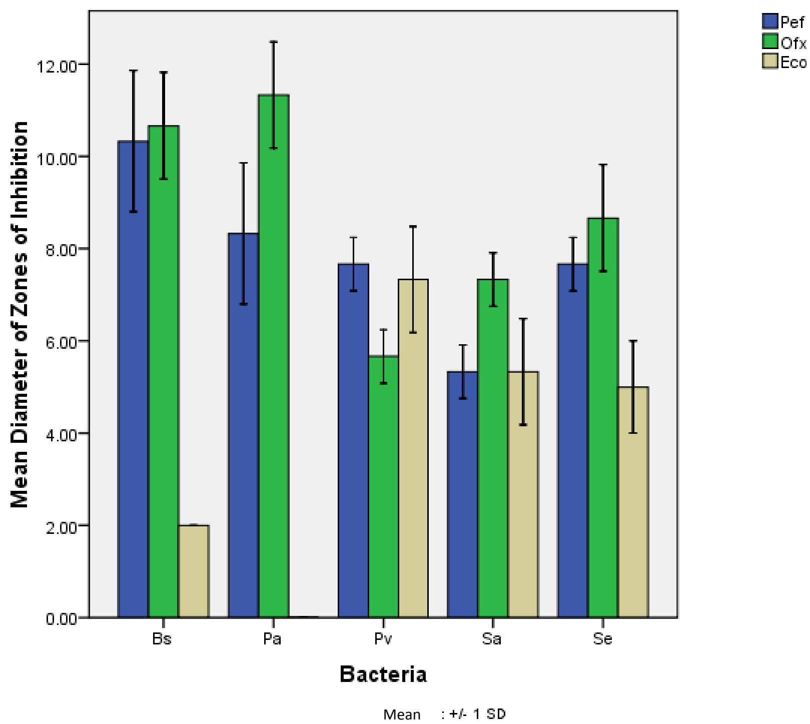

Figure 3. Comparative diameter of zones of inhibition of commercial antibiotics and ethanol extract of *Chromolena odorata* on bacterial isolates. Legend: Bs- *Bacillus subtilis*, Pa- *Pseudomonas aeruginosa.* Pv- *Proteus vulgaris,* Sa- *Staphylococcus aureus,* Se- *Staphylococcus epidermidis*; Pef- Pefloxacin; ofx- ofloxacin; Cpx- Ciprofloxacin; Eco- *Chromolena odorata.*

who swim in such pools be infected or affected CDC (2000) in an attempt to answer these questions stated that certain factors such as the dose of the organism present, health status of the swimmer, type of chemical sterilant used are factors to be checked before concluding the answers.

S. aureus having the highest percentage of occurrence results from its abundance on the body of human which is its normal flora Valarmathi et al. *(*2013). The presence of *S. aureus* on the skin might not pose any threat as it is the normal flora of human skin, but can pose major threat when it finds its way into the mucosal region of the skin where it can cause cellulitis, impetigo septicemia etc (Vos, 2012; Kumar et al., 2007). Consequent release of these bacteria can results to Pelvic Inflammatory Disease (PID) (Bartlett et al., 2013) and Urinary Tracts Infections (UTI) (Beerepoot et al., 2012) which are mostly associated with *Pseudomonas* sp, *Klebsiella* sp, *Escherichia coli* and S*. aureus* most especially among females due to their short clitoris.

The relevance of the standard antibiotics used in this research is that these hotels and other hotels with commercial swimming pool can and should employ the liquid forms of some of these antibiotics in treatment of the water after chlorination. According to Bartlett et al., 2013, there is need for hotels to have swimming pool water quality control officers who are microbiologists that will constantly carry out isolation and susceptibility testing of isolates from these pools to enhance constant treatments of these pools. This will prevent further contamination and dissemination of some of these pathogenic bacteria.

The inhibitory evaluation of ethanol extract of *C. odorata* on the bacterial isolates showed that the extract had its highest inhibitory effect on *P. vulgaris*, *S. aureus, B. subtilis* and *S. epidermidis*. The inhibitory activity against *S. aureus* by the ethanolic extract of *C. odorata* is in accordance with the report of Mbajiuka et al. (2014); however, *C. odorata* was resisted by *P. aeruginosa,* which is in agreement with the result obtained by Rojas et al. (2006) and Nascimento et al. (2010), who found various plant extracts resisted by *Pseudomonas* sp. But this is not in agreement with Srisuda et al. (2016) who reported that the ethanolic extract of *C. odorata* as inhibitory effect on *P. aeruginosa.* Variation in the antibacterial efficacy on *Pseudomonas* sp may be due to genetic make-up of the different strains of *Pseudomonas* sp. Whereas, antibiotics such as ofloxacin, ciprofloxacin which are used as positive control exert the highest inhibitory effect on all the bacteria isolated and this is in agreement with the result obtained by Donlan (2002).

CONCLUSION AND RECOMMENDATION

These results have shown that these swimming pools house a variety of microorganisms, some of which are pathogenic and should therefore be disinfected on regular basis to prevent dissemination of these bacteria by swimmers. Swimmers suspected to be infected should not be permitted to swim; therefore medical records should be obtained before anyone is allowed to use the swimming pools. There is absence of feacal indicators such as *E. coli* and this may be that the focus of the hotels management is only eradication of feacal indicators from the swimming pool, whereas there could be other life threatening bacteria in the pools. In view of this, attention should also been drawn to eradication of other pathogenic and resistant bacteria from the swimming pools. *C. odorata* has also been noted for its antibacterial potentials and can then be further developed as an alternative therapy as many pathogens are becoming resistant to conventional antibiotics and some of these antibiotics have various side effects to human being.

Conflict of interests

The authors have not declared any conflict of interest.

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