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Antibacterial activities of *Polyalthia longifolia* leaf extracts on multiple antibiotic-resistant bacteria isolated from hospital fomites in Akure, Nigeria

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Abstract. *Babatunde OJ, Ogundare AO, Adebolu TT. 2023. Antibacterial activities of* Polyalthia longifolia *leaf extracts on multiple antibiotic-resistant bacteria isolated from hospital fomites in Akure, Nigeria. Nusantara Bioscience 15: 149-160. Polyalthia longifolia* (Sonn.), an ornamental plant, is said to be therapeutic while searching for new medications to treat infections caused by multiple Antibiotic-Resistant Bacteria (MAR). This plant's leaves were investigated for prospective antibacterial activity against MAR isolated from fomites in selected hospitals in Akure and their pharmacological properties. Standard microbiological methods were used to isolate and identify bacteria from fomites. Disc diffusion was performed to test their sensitivity to conventional antibiotics and *P. longifolia* leaf extracts made with ethanol and water. Ciprotab® was used as the control during the antibacterial assay. Therefore, GC-MS analysis was carried out using standard methods to identify the chemicals in the plant leaf extracts. *Staphylococcus aureus* (29.17%), *Streptococcus pyogenes* (20.83%), *Pseudomonas aeruginosa* (14.28%), *Escherichia coli* (14.28%), *Salmonella typhi* (12.5%) and *Klebsiella pneumoniae* (8.33%) were the bacterial species isolated from the fomites sampled in this study. Crude ethanol leaf extract of *P. longifolia* (100mg/mL) inhibited the growth of these organisms with the greatest effect on *P. aeruginosa* with a value of 23.83±0.44 mm, which is superior to that medicated by the control antibiotic (ciprofloxacin). The GC-MS analysis of the purified leaf extracts of *P. longifolia* revealed the presence of bioactive compounds such as n-hexadecanoic acid and phytol, among others. The study revealed that the leaf extracts of *P. longifolia* can inhibit the growth of the isolated MAR from fomites with an added cidal effect, and the inhibition increase with the increase in concentration and exposure time.

Keywords: Fomites, hospital, MAR bacteria, *Polyalthia longifolia*

INTRODUCTION

Over the past ten years, there has been a significant increase in the focus placed on the role that contaminated hospital surfaces and environments play in transmitting Hospital-Acquired Infections (HAIs) (Sen and Sen 2020). According to Walji et al. (2021), a surface that has been polluted could become a reservoir for various pathogens; as a result, it has the potential to be a significant source of HAI spreading. Furthermore, these pathogens can remain infectious on fomites for weeks, even in extreme circumstances. They can survive for months due to the generation of spores, which could increase the risk to the hospital patients exposed to them (Dancer and Kramer 2019).

The development of antibiotic resistance among the bacterial pathogens responsible for community- and hospital-acquired illnesses is a major concern for public health (CDCP 2013). Furthermore, nosocomial infections are more frequently associated with the presence of Multiple Antibiotic-Resistant (MAR) organisms, and these infections contribute to an increase in patients' mortality and morbidity (Peters et al. 2019; Dunachie et al. 2020). There have also been reports of organisms responsible for nosocomial infections exhibiting resistance to several medicines, making it harder to treat bacterial infections. In addition, new patients who move into a room previously occupied by a patient with MAR infections are at an increased risk of contracting these pathogens because they are more likely to come into contact with them (Penrice et al. 2021).

Creating new antimicrobial agents is one of the actions planned to slow the ongoing spread of antibiotic resistance (Persaud et al. 2019; Babatunde et al. 2022). According to the Global Health Organization (WHO) estimate, more than 75 percent of the globe's population relies entirely or partially on herbal medicines for their health care (WHO 2013). As a result, plants have been utilized in developing pharmaceuticals and have served as the basis for various sophisticated conventional medical systems (Akinjogunla et al. 2011). However, according to Odugbemi (2006), some of the recognized medicinal plants have been utilized in an antique manner, which means that the scientific community has not evaluated them. These plants have traditionally been used to treat fever symptoms, cough, and stomach issues. However, only several medicinal plants like *Allium sativum* (Adebolu et al. 2011), *Vernonia amygdalina* (Ogundare 2011), *Ocimum gratissimum* (Bhavani et al. 2019), *Andrographis paniculata* (Isunu et al. 2023), among others, have been evaluated scientifically.

Moreover, *P. longifolia,* which has been selected in this study, has been reported by some researchers to have medicinal values (Thenmozhi and Sivaraj 2010; Nagore et al. 2021; Firdous et al. 2022). On the other hand, there is a paucity of data about their antibacterial efficacy against microorganisms resistant to MAR. Therefore, the purpose of this study was to investigate the level of antibacterial activity exhibited by the ethanol and aqueous leaf extracts of *P. longifolia* on MAR bacteria isolated from hospital fomites in Akure, Nigeria. In addition, an investigation of the chemical ingredients of the plant's components responsible for its antibacterial properties was also carried out.

MATERIAL AND METHODS

Isolation and identification of bacteria

Therefore, a total of 88 Swab samples were obtained from door knobs, beddings, laboratory gowns, hospital stretchers, bedside lockers, toilet seats, and railings from different hospitals in Akure, Ondo State. The hospitals include the University of Medical Sciences Teaching Hospital (UNIMEDTH), ChristBay Consultant (CBC) Hospital, Unity Clinic (UC), Comprehensive Health Centre (CHC), Arakale, and FUTA Health Centre (FHC). In addition, the isolation of bacteria was carried out as described by Aderanti et al. (2019), while the presumptive identification of the isolated bacteria was carried out using the biochemical characterization described by Olutiola et al. (2000).

Standardization of inoculum

Standardization of the culture to 0.5 McFarland's standard (10^6 CFU/mL) was done as described by Isunu et al. (2022).

Antibiotic sensitivity testing

The antibiotic sensitivity test was performed using the method described by Bauer (1966). First, the zone of inhibition's diameter was measured with a calibrated ruler and then compared with the Clinical and Laboratory Standards Institute's for their sensitivity or resistance standards (CLSI 2017).

Collection of plant materials

The leaves of *P. longifolia* were collected at the Obanla campus of the Federal University of Technology, Akure (FUTA), Nigeria, where they were planted for ornamental purposes. The leaves were after that authenticated at the Department of Crop, Soil, and Pest Management, FUTA.

Preparation of plant extracts

The air-dried leaf samples of *P. longifolia* were powdered using a Marlex blender (Electroline model IS 4780, CM/L 7902804). First, the powdered plant materials were extracted using ethanol, hot water, and cold water. Next, 100 g of the ground samples were weighed into three containers and labeled according to their solvents. Afterward, 1,000 mL of each solvent was added to respective containers, covered, and shaken intermittently for 72 hours. Next, the Solvents were drained using a muslin cloth and filtered with Whatman No. 1 filter paper. Finally, the semi-solid extracts were obtained using a rotary evaporator (RE-52A Union Laboratories, England).

Phytochemical analysis

The leaf extracts of *P. longifolia* were analyzed for the presence or absence of different phytochemicals such as alkaloids, saponins, tannins, anthraquinone, flavonoids, steroids, terpenoids, and cardiac glycosides using standard methods described by AOAC (2011).

Antimicrobial activity of *Polyalthia longifolia*

The assay for the antibacterial activity of *P. longifolia* extracts was carried out as described by Isunu et al. (2022) with modifications. First, the extracts' reconstitution was done according to the various concentration intended for use in this study. For instance, to get 100 mg/mL of each extract, 1 g was dissolved in 10 ml of 30% Dimethyl Sulfoxide (DMSO). Sterile perforated filter papers were then impregnated with the reconstituted extracts and placed accordingly on Mueller-Hinton agar plates streaked with the test organisms. Positive control was maintained with 2mg/mL of Ciprotab ®. The plates were then incubated for 18 hours at 37 °C, and the diameter of inhibition zones was measured in mm.

Determination of Minimum Inhibitory Concentration (MIC)

This assay used the broth dilution method with peptone water broth. A series of test tubes containing different concentrations of the extracts of *P. longifolia*, ranging from 6.25 mg/mL to 100 mg/mL, were inoculated with the standardized bacteria and incubated for 18-24 hours. The lowest concentration of *P. longifolia* extracts dissolved in the broth medium and inoculated with no visual turbidity was recorded as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC of *P. longifolia* extracts was determined using the method developed by Bosso and Innalegwu (2018). The test tubes from the MIC test that did not show visible growth were aseptically inoculated on different sterile Muller-Hinton agar plates and incubated at 37 °C for 24 hours. The MBC was chosen as the lowest extract concentration, resulting in no visible growth of the bacterial isolates on the plate.

Determination of the killing rate of extracts

Using the pour plate technique described by Ogundare (2011), the killing rate of ethanol extract on *P. longifolia* leaves on the bacterial isolates was determined at 0, 10, 20, 30, 60, 120, 240, 360, and 480 mins, after which observation was made for microbial growth and colony count was carried out.

Identification of chemical compounds using GC/MS

The method of Olusola-Makinde et al. (2021) was used to evaluate the chemical components and their percentage

abundance using a Varian GC – MS equipment (Varian 4000 mass spectrometer, USA) alongside a mass spectrometer (MS) 3000 equipped with Agilent MS capillary column (30 m × 0.25 mm, i.e., film thickness).

Statistical analysis

Data obtained in this study were subjected to a one-way Analysis of Variance (ANOVA), and differences between means were compared by Duncan's New Multiple Range Test at a 95% confidence interval using Statistical Package for Social Sciences (SPSS) version 26.0.

RESULTS AND DISCUSSION

Identification of bacterial isolates

The biochemical characterization tests revealed the identity of the bacteria isolated from fomites in this study. The identified bacteria include *Staphylococcus aureus* (n=14), *Streptococcus pyogenes* (n=10), *Escherichia coli* (n=7), *Pseudomonas aeruginosa* (n=7), *Salmonella typhi* (n=6) and *Klebsiella pneumoniae* (n=4) as shown in Table 1.

Rate of occurrence of bacteria isolated from hospital fomites

The percentage occurrence of the bacteria isolated from the hospital fomites. The *S. aureus* was observed as the most frequently isolated bacteria (29.17%), followed by *S. pyogenes* (20.83%), while *K. pneumoniae* (8.33%) was the least frequently occurring among the isolates obtained in this study (Figure 1).

The percentage occurrence of the bacterial isolates from each hospital sampled is shown in Figure 2. Each bacterial isolate was found to be more frequently occurring at UNIMEDTH. On the contrary, at Christ Bay Clinic and Unity Clinic, *S. pyogenes* had a 0% rate of occurrence. However, the same bacteria had 70%, 20%, and 10% at UNIMEDTH, Comprehensive Health Centre, and FUTA Health Centre, respectively.

The occurrence rate of bacteria on each fomite sampled is displayed in Figure 3. The *E. coli* (71.43%) was found to occur more frequently on toilet seats. *S. aureus* (50%), *K. pneumoniae* (50%), and *P. aeruginosa* (28.57%) were found to be more frequently occurring on beds, door knobs, and railings, respectively.

In healthcare settings, particularly hospitals, antimicrobial-resistant bacteria are becoming a growing concern. These microorganisms can survive on fomites, surfaces, or objects that transmit infections. For example, there are frequent fomites in hospitals, doorknobs, bed rails, and medical equipment. Patients can contract antimicrobial-resistant bacteria in contact with these contaminated fomites (Dalton et al. 2020). Antimicrobial-resistant bacteria can cause hospital-acquired infections that are severe and difficult to treat. In addition, patients already weakened due to illness or injury may be more susceptible to these infections, resulting in longer hospital stays, higher medical costs, and even death (Brown et al. 2020).

This study analyzed the distribution and antimicrobial resistance pattern of bacteria isolated from hospital fomites in Akure, Nigeria. In addition, the frequency of occurrence of these bacterial pathogens was estimated. The bacteria isolated in this study from hospital fomites were *S. aureus*, *S. pyogenes*, *K. pneumoniae*, *P. aeruginosa*, *S. typhi,* and *E. coli*. In addition, the frequency of occurrence of these bacterial pathogens was estimated. These findings are similar to the report by Moges et al. (2014) in which *Klebsiella* sp., *Pseudomonas* sp., *E. coli*, *Citrobacter* sp., *S. aureus,* and two species of *Shigella* were isolated from hospital environments. However, although most of the bacteria isolated in this study were similar to what they identified, the percentage occurrence of each differed. Similarly, a study by Prasad et al. (2018) presented the presence of multiple antibiotic-resistant *P. aeruginosa*, *S. pyogenes*, *E. coli*, *S. aureus*, *S. typhi,* and *K. pneumonia* in a hospital environment.

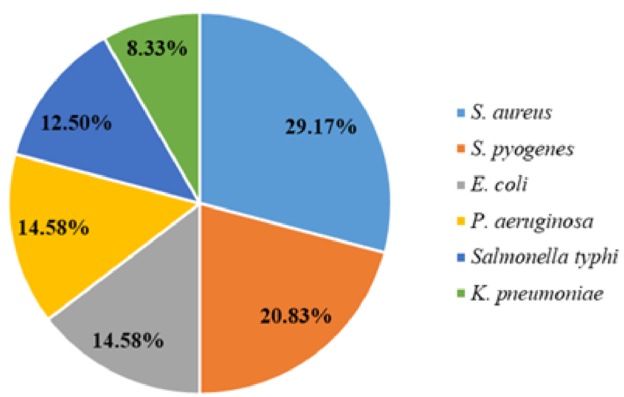

Figure 1. Percentage occurrence of bacteria isolated from Hospital fomites

Table 1. Biochemical characterization of bacterial isolates

Shape	GR	CA	CI	CO	MO	H_2S	UR	IN	OX	FU	GU	GA	SU	LA	MA	MN	GAS	Probable organisms
Cocci	+	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	-	*Staphylococcus aureus*
Rod	-	+	-	-	+	-	-	+	-	-	+	+	+	+	-	+	+	*Escherichia coli*
Cocci	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	*Streptococcus pyogenes*
Rod	-	+	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+	*Klebsiella pneumoniae*
Rod	-	+	+	-	+	-	-	-	+	-	-	-	-	-	-	+	-	*Pseudomonas aeruginosa*
Rod	-	+	-	-	+	+	-	-	-	-	+	-	-	-	+	+	-	*Salmonella typhi*

Note: GR: Gram's reaction, CA: Catalase, CI: Citrase, CO: Coagulase, MO: Motility, H2S: Hydrogen Sulphide, UR: Urease, IN: Indole, OX: Oxidase, FU: Fructose, GA: Galactose, GU: Glucose, SU: Sucrose, LA: Lactose, MA: Maltose, MN: Mannitol

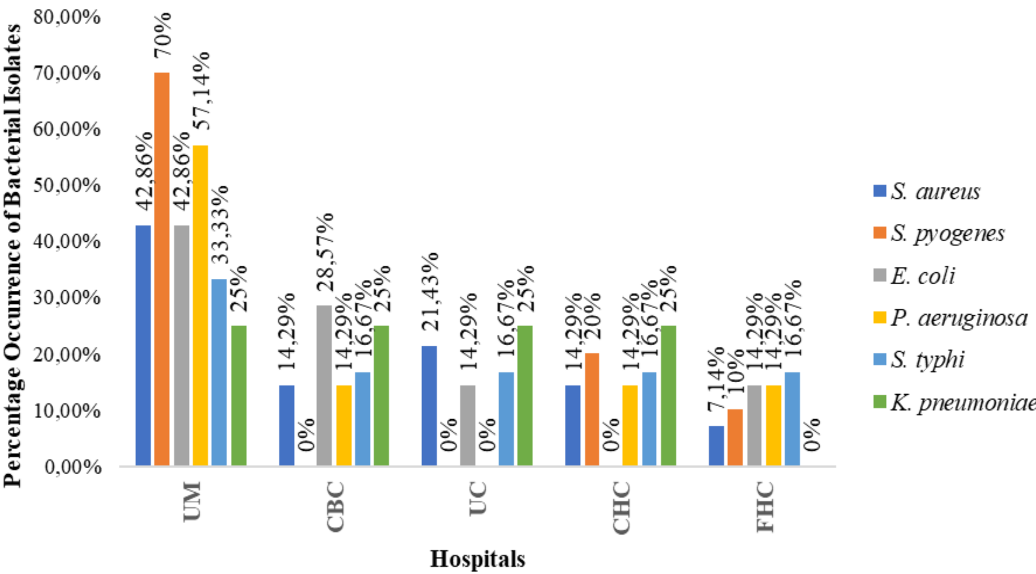

Figure 2. Distribution of bacteria isolated across different hospitals. Note: CBC: Christ Bay Clinic, CHC: Comprehensive Health Centre, FHC: FUTA Health Centre, UC: Unity Clinic, UNIMEDTH: University of Medical Sciences Teaching Hosptial

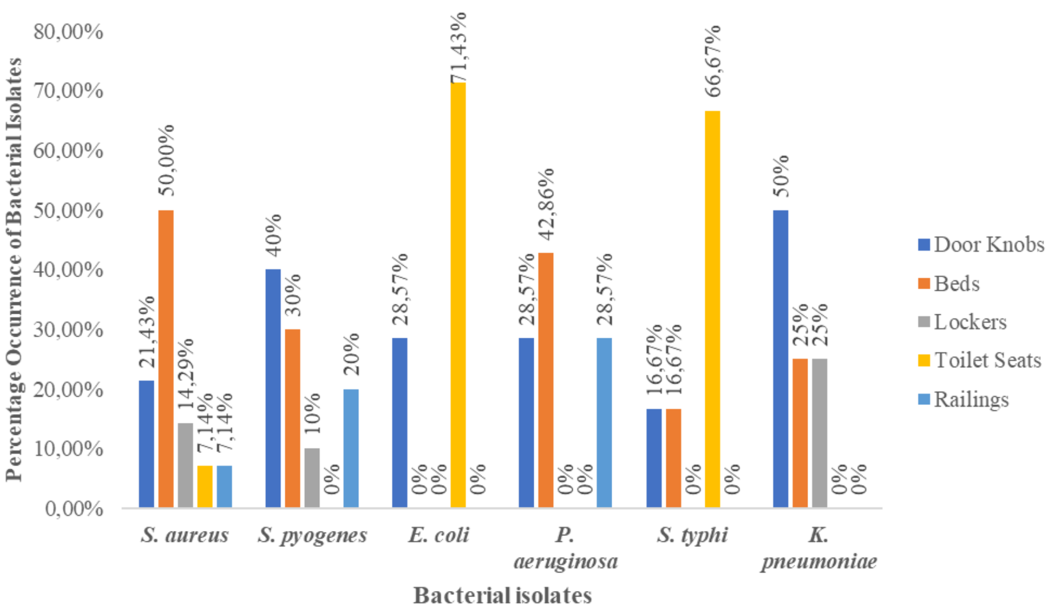

Figure 3. Distribution of bacteria isolated across different fomites

Antibiotic sensitivity pattern of bacterial isolates

The antibiotic sensitivity test results are shown in Tables 2 and 3 for Gram-negative and Gram-positive bacteria, respectively. All the bacterial isolates were more susceptible to ciprofloxacin, with the inhibition zone diameter ranging from 13.50±0.29 mm against *S. aureus* to 29.33±1.45 mm against *K. pneumoniae*. In addition, the isolates were heavily resistant to augmentin and amoxicillin except for *K. pneumoniae,* with an inhibition zone of 14.17±0.73 mm by amoxicillin.

Most isolated bacteria demonstrated multiple antibiotic resistance to the conventional antibiotics used in this study. This is in line with the report of Oliveira and Damasceno (2010), who identified the antibiotic-resistance pattern of bacteria from hospital environments due to the heavy presence of antimicrobial agents in such environments, leading the bacteria to develop resistance. The high rate of resistance demonstrated by the isolated bacteria to antibiotics further lends credence to the fact that almost all known bacterial species are fast becoming resistant to available antibiotics.

Table 2. Antibiotic sensitivity profile of gram-negative bacterial isolates

Isolates	PEF	OFX	S	SXT	CH	SP	CPX	AM	AU	GN
E. coli	20.67±0.33^{cd}	18.83±0.60^{c}	12.50±0.76^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	21.50±0.50^{b}	0.00±0.00^{a}	0.00±0.00^{a}	21.17±1.09c
K. pneumoniae	29.67±0.88^{e}	27.83±0.73^{de}	18.83±1.74^{c}	21.67±0.33^{c}	27.83±0.73^{de}	30.00±0.58^{e}	29.33±1.45^{e}	14.17±0.73^{b}	0.00±0.00^{a}	23.33±0.67^{cd}
P. aeruginosa	17.83±1.01^{b}	17.83±0.17^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	21.17±0.60^{c}	0.00±0.00^{a}	0.00±0.00^{a}	19.67±0.88^{bc}
S. typhi	21.83±0.17^{d}	19.17±0.93^{c}	13.33±0.17^{b}	0.00±0.00^{a}	0.00±0.00^{a}	14.5±0.50^{b}	26.17±1.09^{e}	0.00±0.00^{a}	0.00±0.00^{a}	22.17±0.17^{d}

Note: PEF: Pefloxacin (30µg), OFX: Ofloxacin (10µg), S: Streptomycin (30µg), SXT: Septrin (30µg), CH: Chloramphenicol (30µg), SP: Sparfloxacin (10µg), CPX: Ciprofloxacin (30µg), AM: Amoxacillin (30µg), AU: Augmentin (10µg), GN: Gentamycin (30µg). Values are presented as mean±SE of triplicates; values in the same row carrying the same superscript are not significantly different ($p < 0.05$) according to Duncan's New Multiple Range Test

Table 3. Antibiotic sensitivity profile of gram-positive bacterial isolates

Isolates	PEF	GN	APX	Z	AM	R	CPX	S	SXT	E
S. aureus	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	13.50±0.29^{b}	21.33±0.33^{c}	31.67±0.33^{d}	0.00±0.00^{a}
S. pyogenes	20.17±0.17^{d}	20.17±0.44^{d}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	20.33±0.17^{d}	16.00±0.00^{c}	0.00±0.00^{a}	13.33±0.33^{b}

Note: PEF: Pefloxacin (10µg), GN: Gentamycin (10µg), APX: Ampiclox (30µg), Z: Zinnacef (20µg), AM: Amoxacillin (30µg), R: Rocephin (25µg), CPX: Ciprofloxacin (10µg), S: Streptomycin (30µg), SXT: Septrin (30µg), E: Erythomycin (10µg). Values are presented as mean±SE of triplicates; values in the same row carrying the same superscript are not significantly different ($p < 0.05$) according to Duncan's New Multiple Range Test

Phytochemical constituents of the extracts

The qualitative phytochemical analysis revealed that both flavonoids and steroids are absent while confirming the presence of saponins, tannins, anthraquinones, terpenoids, alkaloids, and cardiac glycosides, as shown in Table 4. Phytochemicals such as saponins, tannins, anthraquinones, terpenoids, alkaloids, and cardiac glycosides were found present in the various extracts. Jothy et al. (2013) reported a similar observation about the phytochemical composition of the plant. The synthesis of secondary metabolites with antibacterial properties is an important defense mechanism for plants, and these chemicals have potential medical and agricultural benefits (O'Bryan et al. 2015).

Antimicrobial activity of *Polyalthia longifolia*

The antibacterial activities of *P. longifolia* leave extracts at 100 mg/mL showed that crude ethanol extract produced the widest zone of inhibition against *P. aeruginosa* with a value of 23.83±0.44 mm. In contrast, the control against the same isolate produced a zone of inhibition of 0.00±0.00 mm, indicating that the crude ethanol extract is more effective than the control against the isolate (Table 5). At a concentration of 25 mg/mL, the activities of the extracts were reduced, as shown in Table 6. The control antimicrobial agent was maintained at 2 mg/mL throughout the experiment.

Leaves extracts of *P. longifolia* had antibacterial effects on the isolates at various concentrations. There was a significant difference (at $p < 0.05$) in the activities of the various extracts against the bacterial isolates by comparing the mean values of the inhibition zones they mediated. Additionally, the differences were significant when these values were compared to the mean values of inhibitory zones mediated by the control antibiotic.

This study's ethanol extract of P. longifolia leaves is more antibacterial than hot and cold water extracts against the test isolates. At 100 mg/mL concentration, the ethanol extract mediated an inhibition zone of 18.17±0.17 mm against *S. typhi,* while the cold water extract produced the smallest zone of 12.00±0.00 mm. The control was, however, more effective against the isolate with a mean inhibition zone of 24.50±0.76 mm. Similarly, the control antibiotic mediated a wider inhibition zone against *S. aureus*, *K. pneumoniae*, *E. coli,* and *S. pyogenes* with mean inhibitory values of 28.17±0.17 mm, 30.33±0.33 mm, 26.50±0.29 mm, and 23.00±0.58 mm, respectively. These values were observed to be greater than that of the ethanol extract with values of 21.67±0.33 mm, 15.00±0.58 mm, 21.50±0.76 mm, and 18.50±0.29 mm, respectively. In contrast, the leaf extracts mediated wider inhibition zones (23.83±0.44 mm, 17.67±0.44 mm, and 14.33±0.33 for ethanol, hot water, and cold water, respectively) while the control antibiotic mediated a zone of inhibition 0.00±0.00 mm indicating a strong resistance. These findings agree with the study by Thenmozhi and Sivaraj (2010), who revealed that the extracts of *P. longifolia* produce significant inhibition zones against the bacterial isolates tested, indicating that they are good antibacterial agents.

Table 4. Qualitative phytochemical constituents of *Polyalthia longifolia* extracts

Phytochemical	Ethanol	Hot water	Cold water
Saponins	+	+	-
Tannins	+	-	-
Flavonoids	-	-	-
Steroids	-	-	-
Terpenoids	+	+	+
Alkaloids	+	+	+
Cardio glycosides	+	+	-
Anthraquinones	+	+	+

Table 5. Antibacterial activity of the various extracts of *Polyalthia longifolia* at 100 mg/mL concentration

Isolates	Crude ethanol (mm)	Hot water (mm)	Cold water (mm)	Control (mm)
S. typhi	18.17±0.17^{b}	12.50±0.29^{a}	12.00±0.00^{a}	24.50±0.76^{c}
S. aureus	21.67±0.33^{c}	18.00±0.58^{b}	15.83±0.17^{a}	28.17±0.17^{d}
P. aeruginosa	23.83±0.44^{c}	17.67±0.44^{bc}	14.33±0.33^{b}	0.00±0.00^{a}
K. pneumoniae	15.00±0.58^{b}	13.83±0.73^{ab}	12.33±0.17^{a}	30.33±0.33^{c}
E. coli	21.50±0.76^{c}	16.50±0.29^{b}	10.67±0.33^{a}	26.50±0.29^{d}
S. pyogenes	18.50±0.29^{b}	14.67±0.33^{a}	14.33±0.17^{a}	23.00±0.58^{c}

Note: Values are presented as mean ± SE of triplicates; values in the same row carrying the same superscript are not significantly different at $p < 0.05$ according to Duncan's new multiple range test. Control: CiproTab (2mg/mL)

Table 6. Antibacterial activity of the various extracts of *Polyalthia longifolia* at 25mg/mL concentration

Isolates	Crude ethanol (mm)	Hot water (mm)	Cold water (mm)	Control (mm)
S. typhi	12.17±0.17^{b}	8.33±0.17^{a}	8.33±0.0.33^{a}	24.50±0.76^{c}
S. aureus	15.00±0.29^{b}	13.67±0.33^{ab}	12.50±0.29^{a}	28.17±0.17^{c}
P. aeruginosa	16.00±0.58^{c}	12.00±0.29^{bc}	10.00±0.00^{b}	0.00±0.00^{a}
K. pneumoniae	10.50±0.29^{c}	7.83±0.17^{b}	0.00±0.00^{a}	30.33±0.33^{d}
E. coli	12.50±0.50^{c}	8.50±0.29^{b}	0.00±0.00^{a}	26.50±0.29^{d}
S. pyogenes	10.67±0.88^{b}	9.33±0.33^{b}	7.67±0.33^{a}	23.00±0.58^{c}

Note: Values are presented as mean ± SE of triplicates; values in the same row carrying the same superscript are not significantly different at $p < 0.05$ according to Duncan's new multiple range test. Control: CiproTab (2mg/mL)

Minimum inhibitory and bactericidal concentration

The MIC values ranged from 6.25 to 12.5 mg/mL for the crude ethanol extract against all the organisms tested. On the other hand, the purified fraction of the ethanol extract, hot water extract, and cold water extract all ranged in MIC values between 6.25 and 25 mg/mL. The lowest MBC value obtained in this study is 6.25 mg/mL, which is that of crude ethanol extract against *P. aeruginosa*. The hot and cold water extracts have an MBC value of 50 mg/mL, recorded against *S. pyogenes* (Tables 7 and 8).

The MIC test conducted for this study showed that ethanol, hot water, and cold water extracts of *P. longifolia* leave possess in vitro antibacterial properties at different concentrations. This validates the findings of Okwulehie et al. (2017) that the antibacterial properties of plant extracts are concentration-dependent. Therefore, the MIC and MBC values of a plant extract are indicators of the extract's antibacterial activity, as Bosso and Innalegwu (2018) stated. In addition, on comparing MIC and MBC values, a plant's ability to fight bacteria is indicated by how low those values are. This investigation showed that the Minimum Effective Concentration (MIC) values were lower than the Minimum Bactericidal Concentration (MBC) values. That indicates the extracts were bacteriostatic at lower doses but bactericidal at higher ones.

The killing rate of bacterial isolates

The killing rate of the organisms by the extracts at 50 mg/mL indicated that the number of viable cells decreased with time (Figures 4-6). The rate at which the extracts were killing the bacteria was investigated, and it was found that the number of viable cells was decreasing throughout the experiment. The number of bacterial cells almost completely disappeared throughout the experiment proves that the extracts have a cidal impact, provided they are exposed to them long enough. These results are consistent with those obtained by Ogundare (2011). Hence, the longer the bacterial cells are exposed to the extracts of *P. longifolia* leaves, the higher the ability of the extract to inhibit some of the bacteria that were previously resistant to the extract at the beginning of the exposure.

Profile of chemical compounds in the ethanol extract of *Polyalthia longifolia*

The GC-MS analysis of the purified extract of *P. longifolia* revealed the presence of bioactive compounds such as n-hexadecanoic acid, phytol, dibutyl phthalate, palmitoleic acid, 1,2-Benzenedicarboxylic acid, Tetradecanoic acid among others. The compounds with their molecular formula and structures are shown in Table 9, while the GC-MS chromatographic spectra are shown in Figure 7.

The GC-MS analysis of the purified ethanol extract of *P. longifolia* revealed the presence of bioactive compounds such as n-hexadecanoic acid, which Sermakkani and Thangapandian (2012) reported having antimicrobial activity. In addition, oleic acid was also detected in the plant, which has been previously reported by Rahdar et al. (2020) to have some antimicrobial properties, thus justifying why the plant exhibited antibacterial activities against the tested bacterial isolates. Furthermore, other compounds detected have been reported to have antibacterial properties. They include 2-Hydroxy-5-methylbenzaldehyde (Sang and Lin 2019), 1, 2-Benzenedicarboxylic acid (Fadipe et al. 2014), Palmitoleic acid (Watanabe et al. 2021), Phytol (Pejin et al. 2014), Squalene (Nazemi et al. 2022) and Dibutyl phthalate (Khatiwora et al. 2012).

Table 7. Minimum Inhibitory Concentration (MIC) value for each extract and isolate

Isolates	Crude ethanol (mg/mL)	Purified ethanol (mg/mL)	Hot water (mg/mL)	Cold water (mg/mL)
Salmonella typhi	12.5	12.5	25	25
Staphylococcus aureus	6.25	25	6.25	6.25
Pseudomonas aeruginosa	6.25	12.5	12.5	12.5
Klebsiella pneumoniae	12.5	6.25	6.25	6.25
Escherichia coli	12.5	6.25	6.25	6.25
Streptococcus pyogenes	12.5	12.5	12.5	25

Table 8. Minimum Bactericidal Concentration (MBC) value for each extract and isolate

Isolates	Crude ethanol (mg/mL)	Purified ethanol (mg/mL)	Hot water (mg/mL)	Cold water (mg/mL)
Salmonella typhi	25	12.5	25	50
Staphylococcus aureus	12.5	25	25	25
Pseudomonas aeruginosa	6.25	12.5	25	25
Klebsiella pneumoniae	25	12.5	25	25
Escherichia coli	25	12.5	12.5	12.5
Streptococcus pyogenes	25	25	50	50

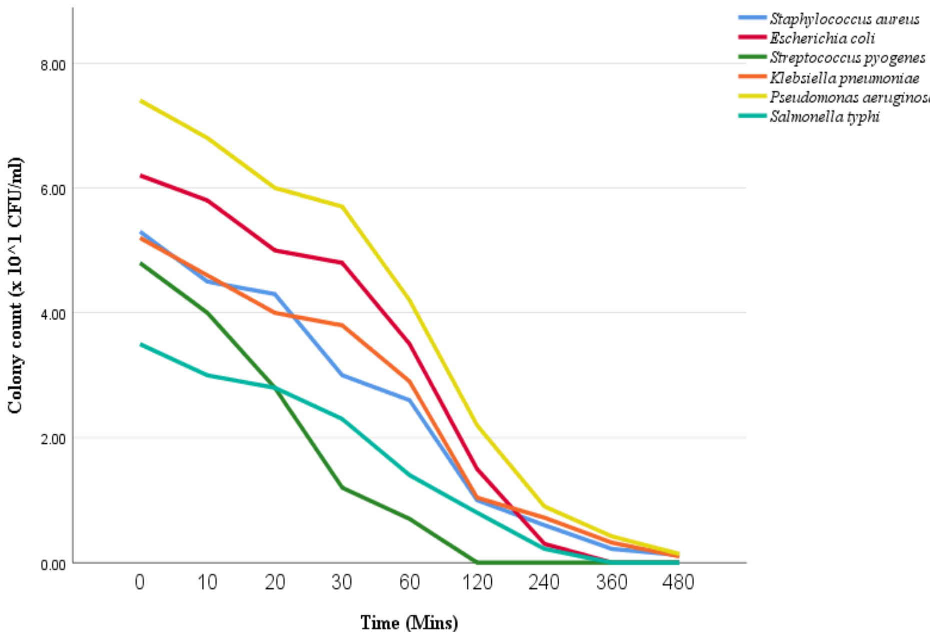

Figure 4. The killing rate of bacterial isolates by 50 mg/mL of crude ethanol extract of *Polyalthia longifolia*

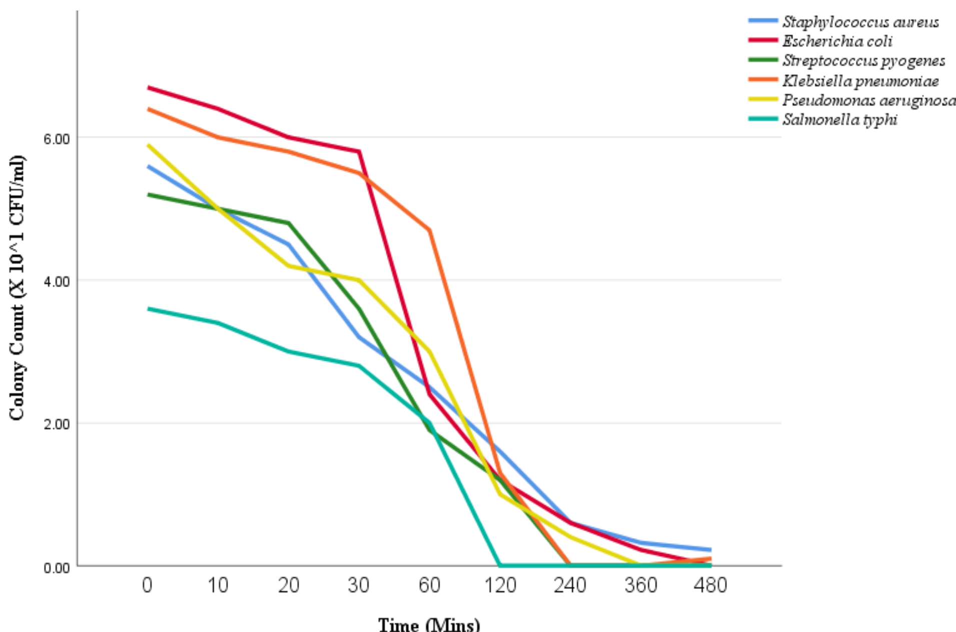

Figure 5. The killing rate of bacterial isolates by 50 mg/mL of hot water extract of *Polyalthia longifolia*

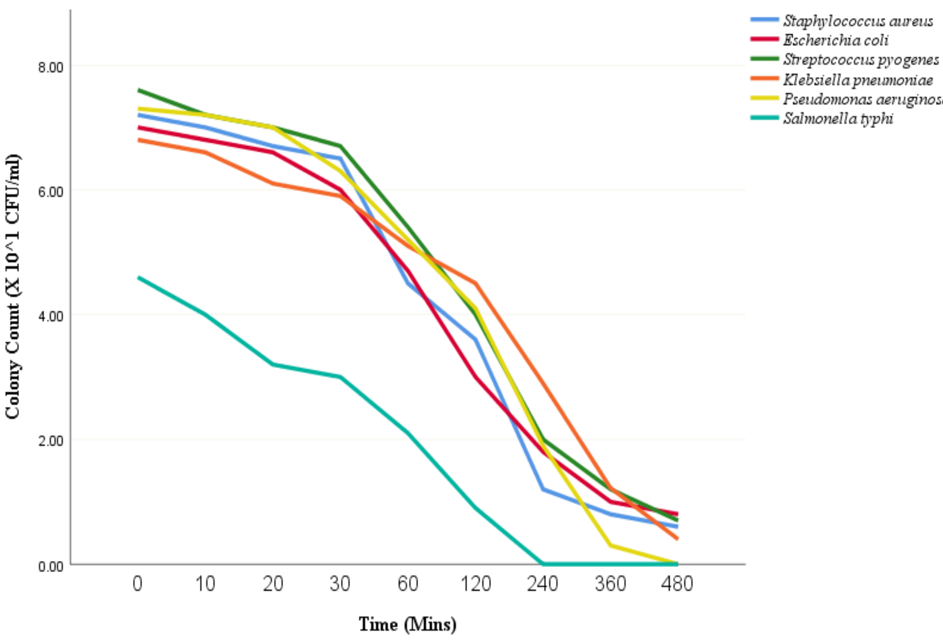

Figure 6. The killing rate of bacterial isolates by 50 mg/mL of cold water extract of *Polyalthia longifolia*

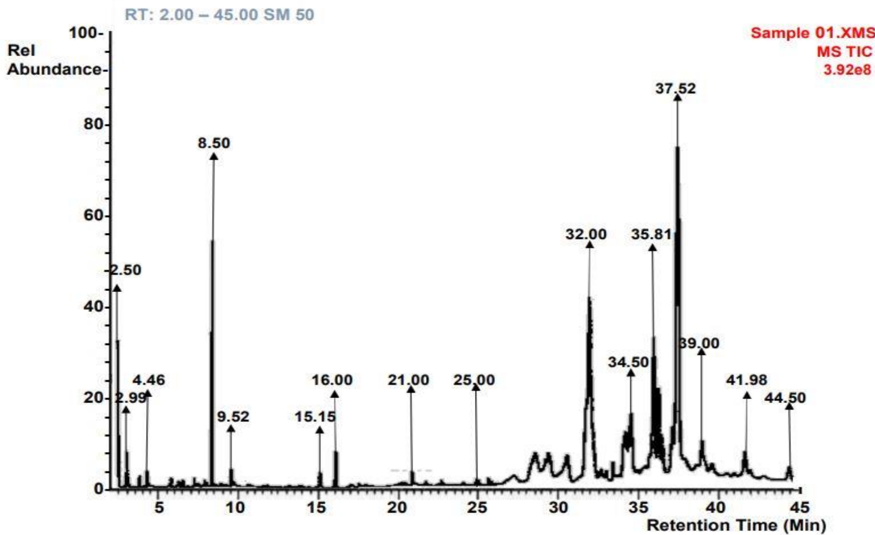

Figure 7. GC-MS chromatographic spectra of ethanol leaf extract of *Polyalthia longifolia*

Table 9. Compounds detected in the Gas Chromatography-Mass Spectrometry of *Polyalthia longifolia* purified ethanol extract

Peak #	RT (Mins)	Compound detected	Mol. formula	MW	Peak area %	Structures
1	2.50	Benzene, 1-ethyl-2-methyl-	C_9H_{12}	120	10.18	
2	2.99	2-Hydroxy-5-methylbenzaldehyde	$C_8H_8O_2$	136	2.73	
3	4.46	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	166	1.24	
4	8.50	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	17.38	
5	9.52	Palmitoleic acid	$C_{16}H_{30}O_2$	254	1.43	
6	15.15	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228	1.30	
7	16.00	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	2.61	
8	21.00	Phytol	$C_{20}H_{40}O$	296	1.37	
9	25.00	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282	1.12	
10	32.00	Oleic Acid	$C_{18}H_{34}O_2$	282	13.66	
11	34.50	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	4.96	
12	35.81	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	10.55	
13	37.52	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	23.59	
14	39.00	cis-11-Eicosenoic acid	$C_{20}H_{38}O_2$	310	3.10	
15	41.98	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	336	2.48	
16	44.50	Squalene	$C_{30}H_{50}$	410	2.30	

In conclusion, this study revealed the presence of multiple antibiotic-resistant bacteria on fomites of the selected hospitals sampled. It further revealed that the different extracts of the leaves of *P. longifolia* used in this study possess some phytochemicals and bioactive compounds that make them good antibacterial agents, as verified using in vitro agar well diffusion assay. Therefore, these extracts might be good sources of more effective drugs to checkmate the activities of MAR on fomites.

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