



Comparative efficacy of *Syzygium cumini* leaf extract and chemical disinfectants against hospital floor isolates: First report of benzalkonium chloride-resistant *Mucor* sp.

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ABSTRACT

The study aimed to isolate and identify organisms present on various floor surfaces within Sathyabama Dental College and General Hospital by standard microbiological procedures and Vitek MS (Biomérieux) and to compare the antibacterial and antifungal effectiveness of *Syzygium cumini* leaf extract as a plant-derived disinfectant with three routinely used chemical disinfectants (CD1, CD2, and CD3) by agar well diffusion method. Furthermore, to determine the antibiotic and antimycotic susceptibility of floor isolates by disc diffusion method. A total of 61 organisms were isolated, including 41 bacteria and 20 fungi. The bacterial isolates were identified as *Staphylococcus* sp., *Bacillus* sp., *Providencia rettgeri*, *Micrococcus* sp., *Pseudomonas aeruginosa*, and *Enterobacter hormaechei*, while fungal isolates included *Mucor* sp., *Alternaria alternata*, *Fusarium oxysporum*, *Penicillium* sp., *Aspergillus flavus*, and *Aspergillus niger*. *Syzygium cumini* extract was effective against all bacterial and fungal isolates, whereas the three chemical disinfectants containing benzalkonium chloride were effective against *Staphylococcus* sp., *Bacillus* sp., and *Micrococcus* sp., but resistant to *P. aeruginosa*, *E. hormaechei*, and *P. rettgeri*. CD1 and CD2 were effective against all fungal isolates except *Mucor* sp., while CD3 showed resistance to all fungi. Gram-positive bacteria were 100% sensitive to Amikacin and Vancomycin and varying resistant to other tested antibiotics. Gram-negative bacteria were 100% resistant to Nitrofurantoin, with varying resistance to other tested antibiotics. Fungal isolates were 100% resistant to Fluconazole, with varying resistance to other antimycotics. *Syzygium cumini* extract showed significant antimicrobial activity against the disinfectant-resistant, antibiotic, and antimycotic-resistant isolates, supporting the potential use of *S. cumini* extract as an alternative, non-toxic, and plant-derived floor disinfectant.

INTRODUCTION

Infectious diseases remain one of the leading causes of morbidity and mortality. The majority of infections are

contracted in a public place [1,2]. Antibiotic resistance has developed as an unprecedented global health threat, driven by its swift and widespread transmission [3]. Antibiotic-resistant pathogens have developed into a major concern in recent years, affecting both community and healthcare-associated infections (HAIs) [4]. The global spread of antibiotic-resistant bacteria reflects an increasing prevalence and highlights the urgent

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need for effective sterilization products to curb the growth and transmission of pathogens [5]. The prevalence of diseases caused by pathogenic filamentous fungi has risen substantially, yet these conditions remain underestimated [6–8]. Fungal contamination in indoor environments can lead to allergic reactions [9], and inhalation of fungal spores, fragments, or metabolites can contribute to respiratory issues, such as asthma, rhinitis, and bronchitis [10]. Molds can exacerbate asthma, cause skin irritation, and headaches, and trigger allergic responses [11,12]. Immunocompromised individuals are particularly at risk, with significant evidence linking mold exposure to various respiratory diseases [6,9]. Several studies highlight the critical role of microbial contamination on surfaces and devices in facilitating pathogen transmission. The efficiency of pathogen spread is contingent upon multiple factors, including the resilience of microorganisms to persist on dry surfaces and the frequency with which contaminated surfaces or devices contact both patients and healthcare personnel [13].

Disinfecting contaminated surfaces effectively lowers the overall infection level while also preventing the dissemination of bacteria to other surfaces [4]. Disinfectants are extensively recognized for eliminating microorganisms from the surfaces of objects and transmission media [14]. They are highly effective in controlling infectious diseases by preventing or eliminating the growth of pathogenic microorganisms within transmission modes [15,16]. They are crucial for maintaining ecological health and safety, with significant applications across various sectors, including healthcare, water treatment and distribution, food processing, agriculture, and other industries [17]. However, the emergence of disinfectant resistance poses a significant threat to public health and safety, as well as to the optimal use of resources, due to the decreased effectiveness of disinfectants [18].

Bacterial resistance to disinfectants plays a critical role in the management of HAIs [19,20]. Microorganisms may display intrinsic resistance to disinfectants, frequently attributed to the low permeability of their cellular structures. Prolonged exposure to disinfectants can further exacerbate microbial resistance through genetic mutations or the acquisition of resistance-conferring genetic elements [13]. Several studies reported that benzalkonium chloride (BC) which is a quaternary ammonium compound class of chemical disinfectants, is resistant against various microorganisms, such as *Staphylococcus aureus* [21], *Escherichia coli* [22], *Pseudomonas aeruginosa* [23], *Acinetobacter baumannii* [24], *Enterobacter* sp. [25], *Listeria monocytogenes* [22], *Aspergillus ochraceus* [26], and *Aspergillus fumigatus* [27]. Regular exposure to commonly used chemical disinfectants increases the risk of chronic obstructive pulmonary disease, asthma, and eye irritation [28,29]. Additionally, there is growing evidence that such exposure may lead to infertility and negatively affect brain development in children [30]. Health workers and individuals who are regularly exposed to these disinfectants are particularly vulnerable, as continuous contact with these chemicals may contribute to long-term health complications [28]. Moreover, it can have detrimental effects on human health, including skin irritation, pruritus, headaches, and dizziness [31]. Residual chemicals left on surfaces often

contain hazardous compounds that are implicated in a range of health issues such as cancer, respiratory disorders, skin irritation, central nervous system dysfunction, and oxidative stress, all of which contribute to a broad spectrum of adverse human health outcomes [28]. Additionally, rainfall facilitates the runoff of disinfectants, leading to contamination of aquatic systems, soil, and atmospheric components. Both direct and indirect discharges of sewage effluents finally enter freshwater bodies such as lakes and rivers, presenting substantial threats to aquatic ecosystems and biodiversity by disrupting ecological stability and endangering the health of wildlife [28,30].

Being an ideal disinfectant, a natural plant-derived disinfectant should possess potent antimicrobial activity and be non-toxic, non-corrosive, cost-effective, user-friendly, and safe for most surfaces. Additionally, it should be safe for skin contact and inhalation and environmentally sustainable [32,33]. *Syzygium cumini* is a large evergreen tree from the Myrtaceae family, known for its wide range of medicinal properties [34,35]. The leaf extract of *S. cumini* has been demonstrated to have significant antibacterial [36,37], antifungal [37,38], and antiviral [39] activities due to the presence of rich bioactive compounds, including tannins, saponins, terpenoids, flavonoids, and phenols, such as sitosterol, betulinic acid, catecholic acid, quercetin, myricetin, and kaempferol [40–42]. Tannins are capable of inhibiting bacterial growth or killing bacteria by binding to bacterial protein cells. This interaction causes protein denaturation, damaging the cell wall and causing lysis [43]. Terpenoids also inhibit bacterial growth [44], while saponins compromise the integrity of cell membranes [45]. Flavonoids form complex bonds with extracellular proteins in the bacterial cell wall, weakening its structure and ultimately causing cell wall breakdown and lysis [43]. Furthermore, *S. cumini* leaf extract is reported to be non-toxic in various cell lines [37,46].

In the present work, environmental bacterial and fungal organisms from different floor surfaces of Sathyabama Dental College and Hospital premises were isolated and identified, and the effectiveness of *S. cumini* leaf extract-derived disinfectant was evaluated toward the antibacterial and antifungal activity in comparison to routinely used chemical disinfectant. Furthermore, the antibiotic and antifungal susceptibility tests against environmental isolates were also determined. This study is the first to report on the antibacterial and antifungal efficacy of *S. cumini* leaf extract against isolates from the environmental floor.

MATERIALS AND METHODS

Isolation of environmental organisms from different surfaces of hospital and college premises

Samples were collected by wiping with a sterile moistened swab from various areas of Sathyabama Dental College and General Hospital (SDCGH) floor surfaces, including the patient waiting area, hospital reception, lecture hall, hospital laboratory, staircase, department of microbiology, auditorium, office, college mess, library, and corridor. The study was approved by the Institutional Biosafety and Ethical Committee (Ref: 331/IRB-IBSEC/SIST Dated 18th October 2023). The collected swabs were inoculated onto the sterile Nutrient agar (NA) plates and Sabouraud dextrose agar (SDA),

respectively. The plates were subsequently incubated at 37°C for 24 hours to facilitate bacterial isolation and at ambient temperature for 72 hours to enable fungal isolation. In order to obtain a pure isolate, individual colonies were picked from each plate and sub-cultured on respective media and then incubated at respective temperatures and days as mentioned earlier [47,48].

Identification of the environmental isolates

Identification of the environmental bacterial isolates was carried out by standard microbiological procedures, such as colony morphological analysis, Gram staining, plated on differential as well as on selective media, and biochemical tests [47]. The isolates were identified based on Bergey's Manual of Systematic Bacteriology and further confirmed by Vitek MS (Biomerieux) [4]. Environmental fungal isolates were identified by colony morphological analysis, Lactophenol cotton blue (LPCB) staining, and confirmed by Vitek MS (Biomerieux) [4,49].

Preparation of extract

Fresh *S. cumini* leaves were collected and thoroughly washed with distilled water, dried, and then ground into fine powder. About 15 g of powder was added to 150 ml of sterile distilled water. Extraction was carried out using the hot percolation method at 60°C for 2 hours. Then, the extract was filtered through Whatman filter paper and then dried overnight in an oven at 40°C–45°C. It was ground into fine powder and stored for further use [37]. Extraction yield % was calculated as the weight of the solvent-free extract/dry weight of the sample $\times 100$ [50].

Chemical disinfectants

The ingredients present in CD-1 are BC solution, water, lauryl alcohol ethoxylate, sodium bicarbonate, cocamidopropyl betaine, perfume, tetrasodium Ethylenediaminetetraacetic acid (EDTA), denatonium benzoate, and CI 47005. CD-2 contains BC solution, water, lauryl alcohol ethoxylate, lauramine oxide, sodium bicarbonate, disodium EDTA, isopropyl alcohol, cocamidopropyl betaine, perfume, and CI 47005. CD-3 contains BC, water, non-ionic surfactant, perfume, preservative, and colorant.

Preparation of the inoculum

The bacterial isolates were inoculated on NA medium and incubated at 37°C for 24 hours. After incubation, 2–3 colonies were taken from the plate and diluted with NB. The turbidity was standardized to the 0.5 McFarland standard to reach a cell density of 10^8 cells/ml. Fungal cultures were inoculated on to SDA medium and incubated at 28°C for 72 hours. Fungal isolates were adjusted to the spore density of 10^6 spores/ml using a spectrophotometer [51].

Antimicrobial activity of *S. cumini* leaf extract and routinely used chemical disinfectant against the bacterial and fungal environmental isolates

The antimicrobial activity of the *S. cumini* extract was compared with three routinely used chemical disinfectants,

which was determined by the agar well diffusion method. The lawn culture was made with the isolated organisms onto the Muller–Hinton agar [47]. Chemical disinfectants were diluted in sterile distilled water based on the instructions provided by the manufacturers. Hundred microliters of *S. cumini* extract (10%), three chemical disinfectants, and distilled water were loaded into the respective wells using a sterile micropipette. The plates were then incubated at 37°C for 24 hours for bacterial cultures and 3 days at room temperature for fungal cultures. Following incubation, the diameter of the inhibition zone was determined. The experiment was conducted in triplicate.

Antibiotic susceptibility test against the bacterial environmental isolates

Antibiotic susceptibility test against the bacterial isolates was done on Muller–Hinton agar by disc diffusion method according to Clinical and Laboratory Standards Institute guidelines [52]. Antibiotics used for Gram-positive isolates were Cephalothin (CEP30), Vancomycin (VA30), Novobiocin (NV30), Erythromycin (E15), Oxytetracycline (O30), Amikacin (AK10), Amoxicillin (AMX10), and Bacitracin (B10). Antibiotics used for Gram-negative isolates were Ciprofloxacin (CIP10), Co-Trimazine (CM25), Kanamycin (K30), Nitrofurantoin (NIT300), Streptomycin (S10), Tetracycline (TE30), Amikacin (AK10), and Carbenicillin (CB100). Lawn cultures of bacterial isolates were inoculated onto Muller Hinton agar (MHA). Respective antibiotics were then placed on the media and incubated at 37°C for 24 hours. The zone of inhibition was measured after incubation. The experiment was conducted in triplicate.

Antifungal susceptibility test against the fungal environmental isolates

Antifungal susceptibility tests against the fungal isolates were also done using the disk diffusion method according to CLSI M51-A [53]. Antimycotic drugs used were Nystatin (NS), Amphotericin B (AP), Clotrimazole (CC), Fluconazole (FLC), Itraconazole, and Ketoconazole (KT). Lawn cultures of fungal isolates were prepared on MHA. Antimycotic drugs were then placed on it and incubated at room temperature for 2–3 days, respectively. Subsequent to incubation, the diameter of the zone of inhibition was determined. The experiment was conducted in triplicate.

Statistical analysis

One-way ANOVA and Tukey's HSD post hoc test were used to analyze the significance level of data (IBM SPSS version 25.0). A p -value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Isolation and identification of the environmental isolates

Totally, 61 environmental organisms were isolated from SDCGH floor surfaces, of which 41 were bacterial isolates and 20 were fungal isolates, as shown in (Fig. 1). The isolated bacteria were characterized by colony morphology, gram staining, and biochemical tests and identified using Bergey's Manual of Systematic Bacteriology and further confirmed

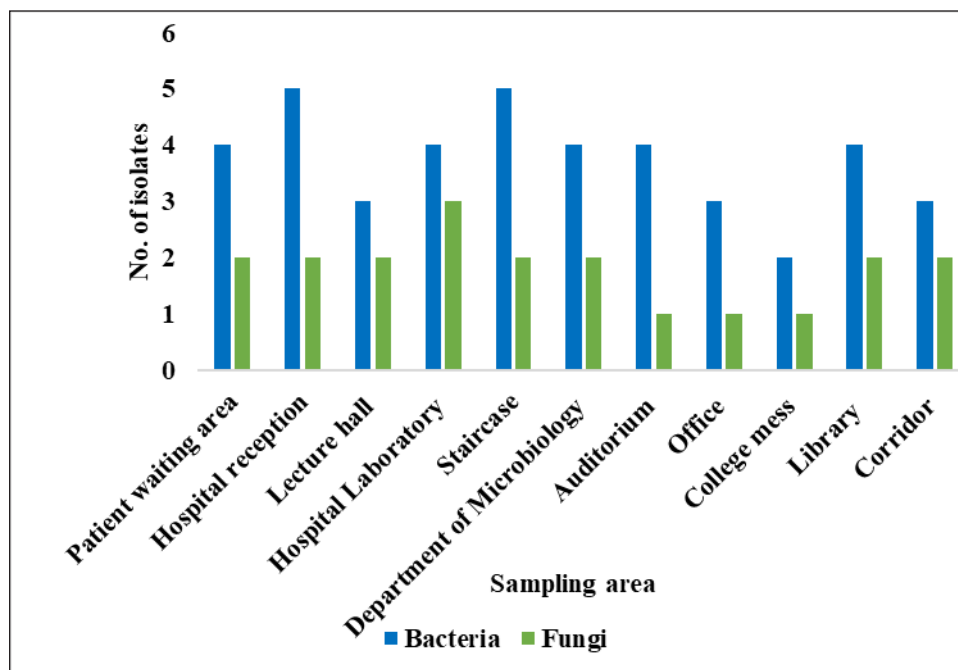


Figure 1. Number of bacteria and fungi isolated from various floors.

by Vitek MS. The identified bacteria were *Staphylococcus* sp., *Bacillus* sp., *Providencia rettgeri*, *Micrococcus* sp., *P. aeruginosa*, and *Enterobacter hormaechei* (Table 1). Among the *Staphylococcus* sp., six of them were *S. aureus*. This is the first study reported on *P. rettgeri* isolated from environmental floors. Fungal isolates were identified by colony morphology, LPCB staining and further confirmed by Vitek MS. The identified fungi were *Mucor* sp., *Alternaria* sp., *Fusarium* sp., *Penicillium* sp., *Aspergillus flavus*, and *Aspergillus niger* (Fig. 2 and Table 2). Palmer and Onifade [48] revealed that *S. aureus*, *Str. pyogenes*, *E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Bacillus cereus*, *A. niger*, *A. fumigatus*, and *Candida albicans* were isolated from hospital environmental surfaces. Similarly, *A. flavus*, *A. niger*, *A. ustus*, *A. fumigatus*, *Rhizopus stolonifera*, *Scopulariopsis* sp., *Trichoderma* sp., *Mucor racemosus*, *Mucor hiemalis*, and *Wallemia* sp. were isolated from carpet and floor dust samples of various indoor environments [54]. *Staphylococcus* sp., *Streptococcus* sp., *Bacillus* sp., *Aspergillus* sp., *Mucor* sp., *Fusarium* sp., *Penicillium* sp., *Candida* sp., *Rhizopus* sp., and *Verticillium* sp. were isolated from the hospital indoor environment [55]. The dominant percentage of bacterial and fungal isolates is shown in (Fig. 3). In the case of bacterial isolates, *Bacillus* sp. was found predominantly, followed by *Staphylococcus* sp., *P. aeruginosa*, *E. hormaechei*, *P. rettgeri*, and *Micrococcus* sp., respectively. In the case of fungal isolates, *A. niger* was the most common fungi, followed by *Penicillium* sp., *Fusarium* sp., *A. flavus*, *Mucor* sp., and *Alternaria* sp. According to Viegas et al. [56], the most prevalent bacteria isolated from the indoor environment were *Micrococcus* sp., followed by *Staphylococcus* sp. and *Neisseria* sp., and in the case of fungal isolates, *Chrysosporium* sp. were prevalently found, followed by *Penicillium* sp. and *Chrysonilia* sp.

Extraction yield

Syzygium cumini leaves were extracted using distilled water as a solvent. The obtained extract was brown in color and crystalline in nature after drying. The extraction yield % of *S. cumini* leaves was calculated using the formula mentioned above, and 9.5% of yield was obtained.

Effect of *S. cumini* leaf extract and routinely used chemical disinfectant against the bacterial and fungal environmental isolates

The antibacterial activity of *S. cumini* extract and three routinely used chemical disinfectants, such as CD1, CD2, and CD3 were determined against the organisms isolated from SDCGH floor surfaces by the agar well diffusion method (Table 3). *Syzygium cumini* extract showed good activity against all the bacterial environmental floor isolates, such as *Staphylococcus* sp., *Bacillus* sp., *Micrococcus* sp., *P. aeruginosa*, *E. hormaechei*, and *P. rettgeri* (Fig. 4). *Syzygium cumini* extract produced a significant zone of inhibition against *Staphylococcus* sp. in the range of (18.07–25.3 mm), *Bacillus* sp. (12.8–20.5 mm), *Micrococcus* sp. (28.87 mm), *P. aeruginosa* (14.13–18.3 mm), *E. hormaechei* (20.33 mm), and *P. rettgeri* (21.83 mm). The significant antibacterial activity of *Syzygium cumini* extract (SCE) may be due to the presence of various secondary metabolites such as tannins, terpenoids, saponins, and flavonoids [40].

BC, the active ingredient of the routinely used chemical disinfectant, was used in the current investigation. In the case of chemical disinfectant, CD1 showed good activity against bacterial isolates, followed by CD2 and CD3. In this study, there was a significant difference ($p < 0.05$ according to Tukey's HSD post hoc test) between the SCE and chemical disinfectants on antibacterial activity against the isolates. CD1

Table 1. Morphological and biochemical tests of bacterial environmental floor isolates.

Sl. No.	Isolate No.	Gram stain	Spore	Motility	Colony morphology	C*	O	I	MR	VP	Ci	NR	Coa	Identified bacteria
1.	PW1	+ rod	+	+	White, smooth, circular, raised, opaque	+	-	-	+	+	+	-	NT	<i>Bacillus</i> sp.
2.	PW2	+ rod	+	+	White, large, opaque, flat, rough, circular with irregular margin	+	+	-	-	+	+	+	NT	<i>Bacillus</i> sp.
3.	PW3	+ cocci**	-	-	Golden yellow, round, smooth, raised, opaque	+	-	-	+	+	+	+	+	<i>Staphylococcus aureus</i>
4.	PW4	- rod	-	+	Green, large, opaque, flat with irregular edge	+	+	-	-	-	+	-	NT	<i>Pseudomonas aeruginosa</i>
5.	L1	+ cocci	-	+	White, round, smooth, convex, opaque	+	-	-	+	+	-	-	-	<i>Staphylococcus</i> sp.
6.	L2	+ rod	+	+	White, large, opaque, flat, rough, circular with irregular margin	+	+	-	-	+	+	+	NT	<i>Bacillus</i> sp.
7.	L3	+ cocci	-	-	White, round, smooth, convex, opaque	+	-	-	+	+	-	-	-	<i>Staphylococcus</i> sp.
8.	L4	- rod	-	+	White, round, smooth, raised, regular	+	-	-	-	+	+	+	NT	<i>Enterobacter hormaechei</i>
9.	RL1	+ rod	+	+	White, large, opaque, flat, rough, circular with irregular margin	+	+	-	-	+	+	+	NT	<i>Bacillus</i> sp.
10.	RL2	+ rod	+	+	White, large, opaque, slightly convex irregular edge	+	-	-	+	-	+	+	NT	<i>Bacillus</i> sp.
11.	RL3	- rod	-	+	Blue-green, large, opaque, flat with irregular edge	+	+	-	-	+	+	+	NT	<i>P. aeruginosa</i>
12.	RL4	+ rod	+	+	Orange, small, translucent, irregular edge	+	-	-	-	+	+	-	NT	<i>Bacillus</i> sp.
13.	RL5	+ cocci**	-	-	Golden yellow, round, smooth, raised, opaque	+	-	-	+	+	+	+	+	<i>S. aureus</i>
14.	LH1	+ cocci*	-	-	Golden yellow, round, smooth, raised, opaque	+	-	-	+	+	+	+	+	<i>S. aureus</i>
15.	LH2	+ rod	+	+	Orange, small, translucent, irregular in shape,	+	-	-	-	+	+	-	NT	<i>Bacillus</i> sp.
16.	LH3	+ rod	+	+	White, smooth, circular, raised, opaque	+	-	-	+	+	+	-	NT	<i>Bacillus</i> sp.
17.	S1	+ cocci	-	-	White, round, smooth, convex, opaque	+	-	-	+	+	-	-	-	<i>Staphylococcus</i> sp.
18.	S2	+ rod	+	+	White, large, opaque, flat, rough, circular with irregular margin	+	+	-	-	+	+	+	NT	<i>Bacillus</i> sp.
19.	S3	+ rod	+	+	White, smooth, circular, raised, opaque	+	-	-	-	+	+	-	NT	<i>Bacillus</i> sp.
20.	S4	+ rod	-	+	Orange, small, translucent, irregular in shape,	+	-	-	-	+	+	-	NT	<i>Bacillus</i> sp.
21.	S5	+ rod	+	+	White, large, opaque, slightly convex irregular edge	+	-	-	+	-	+	+	NT	<i>Bacillus</i> sp.

Continued

Sl. No.	Isolate No.	Gram stain	Spore	Motility	Colony morphology	C*	O	I	MR	VP	Ci	NR	Coa	Identified bacteria
22.	D1	+ cocci **	-	-	Golden yellow, round, smooth, raised, opaque	+	-	-	+	+	+	+	+	<i>S. aureus</i>
23.	D2	+ rod	+	+	White, large, opaque, flat, rough, circular with irregular margin	+	+	-	-	+	+	+	NT	<i>Bacillus</i> sp.
24.	D3	+ rod	+	+	Orange, small, translucent, irregular in shape,	+	-	-	-	+	+	-	NT	<i>Bacillus</i> sp.
25.	D4	+ rod	+	+	White, smooth, circular, raised, opaque	+	-	-	+	+	+	-	NT	<i>Bacillus</i> sp.
26.	LB1	- rod	-	+	Milky white, small, round, opaque, slightly convex	+	-	+	-	+	+	-	NT	<i>Providencia rettgeri</i>
27.	LB2	+ cocci	-	-	White, round, smooth, convex, opaque	+	-	-	+	+	+	-	-	<i>Staphylococcus</i> sp.
28.	LB3	+ rod	+	+	White, large, opaque, slightly convex irregular edge	+	-	-	+	-	+	+	NT	<i>Bacillus</i> sp.
29.	LB4	+ rod	+	+	White, large, opaque, slightly convex irregular edge	+	-	-	+	-	+	+	NT	<i>Bacillus</i> sp.
30.	OF1	+ rod	+	+	White, smooth, circular, raised, opaque	+	-	-	+	+	+	-	NT	<i>Bacillus</i> sp.
31.	OF2	+ rod	+	+	White, large, opaque, flat, rough, circular with irregular margin	+	+	-	-	+	+	+	NT	<i>Bacillus</i> sp.
32.	OF3	+ cocci	-	-	White, round, smooth, convex, opaque	+	-	-	+	+	+	-	-	<i>Staphylococcus</i> sp.
33.	M1	+ cocci	-	-	Pale yellow, round, smooth, convex, opaque	+	-	-	+	+	+	-	-	<i>Staphylococcus</i> sp.
34.	M2	+ rod	+	+	White, large, opaque, flat, rough, circular with irregular margin	+	+	-	-	+	+	+	NT	<i>Bacillus</i> sp.
35.	C1	+ cocci **	-	-	Golden yellow, round, smooth, raised, opaque	+	-	-	+	+	+	+	+	<i>S. aureus</i>
36.	C2	+ rod	+	+	White, large, opaque, slightly convex irregular edge	+	-	-	+	-	+	+	NT	<i>Bacillus</i> sp.
37.	C3	+ rod	+	+	White, large, opaque, flat, rough, circular with irregular margin	+	+	-	-	+	+	+	NT	<i>Bacillus</i> sp.
38.	A1	+ rod	+	+	White, smooth, circular, raised, opaque	+	-	-	+	+	+	-	NT	<i>Bacillus</i> sp.
39.	A2	+ rod	+	+	White, large, opaque, flat, rough, circular with irregular margin	+	+	-	-	+	+	+	NT	<i>Bacillus</i> sp.
40.	A3	+ cocci **	-	-	Golden yellow, round, smooth, raised, opaque	+	-	-	+	+	+	+	+	<i>S. aureus</i>
41.	A4	+ cocci #	-	-	Bright yellow, circular, smooth convex, opaque	+	+	-	-	-	-	+	NT	<i>Micrococcus</i> sp.

A = auditorium; C* = catalase; C = corridor; Ci = citrate; Coa = coagulase; D = Department of Microbiology; I = indole; L = hospital laboratory; LB = library; LH = lecture hall; M = mess; MR = methyl red; NR = nitrate reduction; NT = not tested; O = oxidase; OF = office; PW = patient waiting area; RL = hospital reception; S = staircase; VP = Voges-Proskauer; + = positive, - = negative.

**Cluster arrangement.

#Tetrad arrangement.

Table 2. Colony morphology and microscopic characteristics of fungal environmental floor isolates.

Sl.No.	Isolate No.	Colony morphology on SDA	Microscopic morphology in LPCB stain	Identified fungi
1.	PW1	Fluffy, cottony, white to grey colony	Filamentous hyphae with sporangia	<i>Mucor</i> sp.
2.	PW2	Cottony, flat, white colony with a central orange coloration that spreads throughout the colony	Septate hyphae and sickle-shaped macroconidia	<i>Fusarium</i> sp.
3.	L1	Flat, radially sulcate, grey-green with white periphery colony	Filamentous hyphae with brush-like conidiophore	<i>Penicillium</i> sp.
4.	L2	Dark brown to black conidial head with white periphery	Large conidial head with a globose structure, radiate and biseriate with metulae covering the entire surface	<i>Aspergillus niger</i>
5.	L3	Powdery, yellowish green conidial spores with white periphery	Radiated biseriate conidial head	<i>Aspergillus flavus</i>
6.	RL1	Powdery, yellowish green conidial spores with white periphery	Radiated biseriate conidial head	<i>A. flavus</i>
7.	RL2	Flat, velvety, greyish black with concentric ring around the edge	Obpyriform-shaped conidia with conical beaks	<i>Alternaria alternata</i>
8.	LH1	Powdery, yellowish green conidial spores with white periphery	Radiated biseriate conidial head	<i>A. flavus</i>
9.	LH2	Flat, velvety, radially sulcate, grey-green with white periphery colony	Filamentous hyphae with brush-like conidiophore	<i>Penicillium</i> sp.
10.	S1	Cottony, flat, white colony with a central orange coloration that spreads throughout the colony	Septate hyphae and sickle-shaped macroconidia	<i>Fusarium</i> sp.
11.	S2	Dark brown to black conidial head with white periphery	Large conidial head with a globose structure, radiate and biseriate with metulae covering the entire surface	<i>A. niger</i>
12.	D1	Flat, velvety, radially sulcate, grey-green with white periphery colony	Filamentous hyphae with brush-like conidiophore	<i>Penicillium</i> sp.
13.	D2	Dark brown to black conidial head with white periphery	Large conidial head with a globose structure, radiate and biseriate with metulae covering the entire surface	<i>A. niger</i>
14.	LB1	Flat, velvety, radially sulcate, grey-green with white periphery colony	Filamentous hyphae with brush-like conidiophore	<i>Penicillium</i> sp.
15.	LB2	Fluffy, cottony, white to grey colony	Filamentous hyphae with sporangia	<i>Mucor</i> sp.
16.	OF1	Flat, powdery to velvety, radially sulcate, grey-green with white periphery colony and produced red pigment	Filamentous hyphae with brush-like conidiophore	<i>Penicillium</i> sp.
17.	M1	Dark brown to black conidial head with white periphery	Large conidial head with a globose structure, radiate and biseriate with metulae covering the entire surface	<i>A. niger</i>
18.	C1	Dark brown to black conidial head with white periphery	Large conidial head with a globose structure, radiate and biseriate with metulae covering the entire surface	<i>A. niger</i>
19.	C2	Flat, cottony with abundant white mycelia, pale orange color colony	Septate hyphae and sickle-shaped macroconidia	<i>Fusarium oxysporum</i> <i>complex</i>
20.	A1	Dark brown to black conidial head with white periphery	Large conidial head with a globose structure, radiate and biseriate with metulae covering the entire surface	<i>A. niger</i>

A = auditorium; C = corridor; D = Department of Microbiology; L = hospital laboratory; LB = library; LH = lecture hall; M = mess; OF = office; PW = patient waiting area; RL = hospital reception; S = staircase.

showed antimicrobial activity against bacterial isolates, such as *Staphylococcus* sp., *Bacillus* sp., and *Micrococcus* sp., but no activity was shown against *P. aeruginosa*, *P. rettgeri*, and *E. hormaechei*. Similarly, CD2 showed antimicrobial activity against most of the bacterial isolates, such as *Staphylococcus* sp., *Bacillus* sp., and *Micrococcus* sp.; however, it showed no activity against *P. aeruginosa*, *P. rettgeri*, and *E. hormaechei*. CD3 was found to have the least activity toward the bacterial isolates. It showed antimicrobial activity to some of the *Staphylococcus* sp. and *Bacillus* sp. only; however, no

activity was shown against *P. aeruginosa*, *P. rettgeri*, and *E. hormaechei*. Interestingly, this study showed that *S. cumini* extract was potent against *P. aeruginosa*, *E. hormaechei*, and *P. rettgeri*. However, all the routinely used chemical disinfectants whose active ingredient was BC showed no activity against *P. aeruginosa*, *E. hormaechei* and *P. rettgeri*. Our results were consistent with the previous studies reported that BC does not show activity against *P. aeruginosa* [23,57,58] and *P. rettgeri* [59,60]. It also does not show activity against *Enterobacter* sp. isolated from the hospital environment [25].

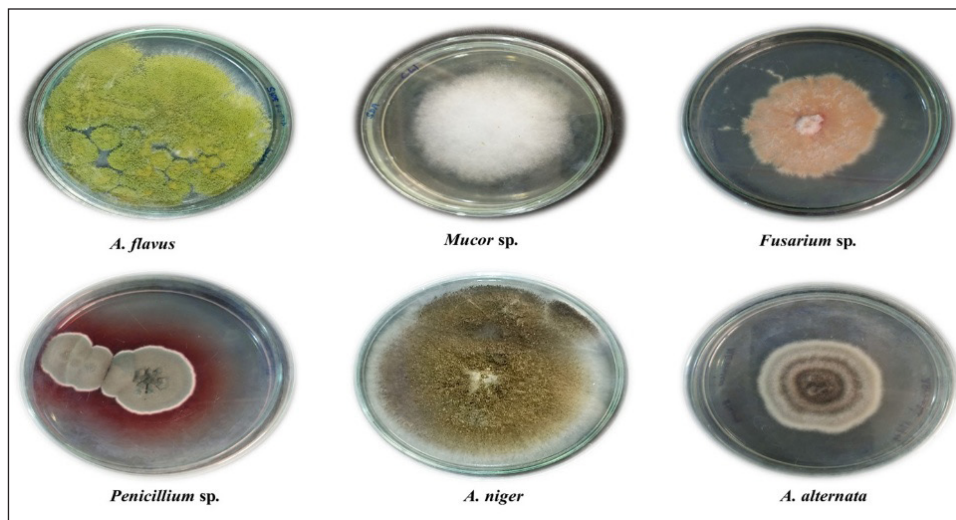


Figure 2. Fungal floor isolates on SDA.

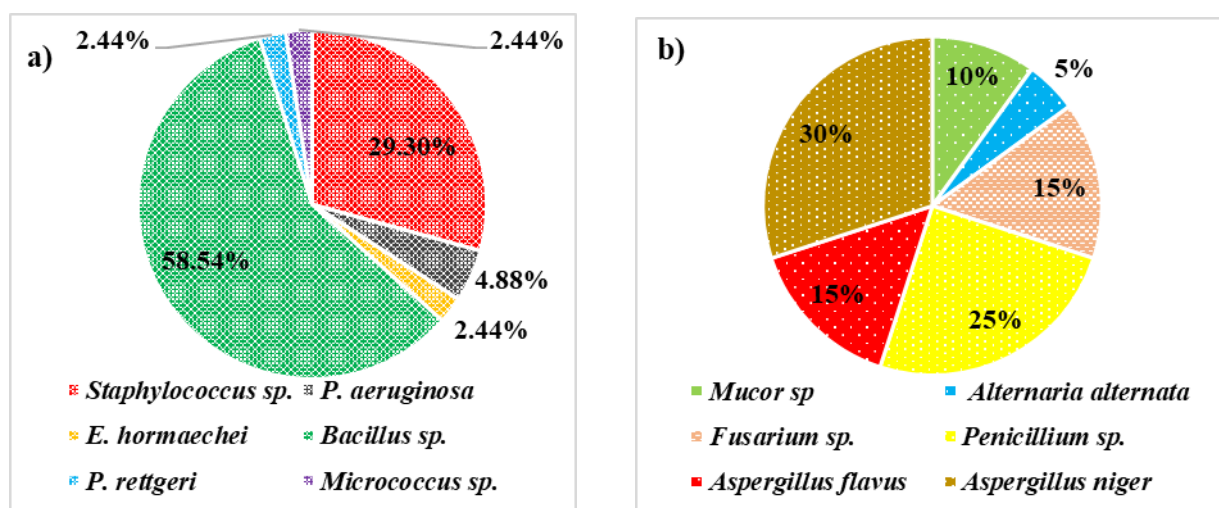


Figure 3. Dominant percentage of a) bacterial isolates and b) fungal isolates.

Microbial innate resistance to disinfectants might be typically associated with the impermeability of their cell membranes, which prevents disinfectant agents from penetrating and affecting the cells [19]. This inherent defense mechanism renders certain microorganisms less susceptible to the action of disinfectants. However, continuous or prolonged exposure to disinfectants can lead to an increase in resistance [61]. This enhancement of resistance occurs through two main mechanisms: genetic mutations within the microorganism’s genome, which may lead to adaptations/alterations that enable survival in the presence of disinfectants, and the acquisition of resistance genes from other microorganisms, often via horizontal gene transfer. These processes contribute to the development and spread of disinfectant resistance, which makes it more challenging to control the microorganisms [13].

The antifungal activity of *S. cumini* extract and three routinely used chemical disinfectants, such as CD1, CD2, and CD3, were also determined against the environmental fungal isolates (Table 4). *Syzygium cumini* extract showed significant activity

against all the environmental fungal isolates, such as *Mucor* sp., *Penicillium* sp., *Fusarium oxysporum*, *Alternaria alternata*, *A. niger*; and *A. flavus* (Fig. 5). *Syzygium cumini* extract exhibited a significant zone of inhibition against *Mucor* sp. in the range of (23–26.33 mm), *Penicillium* sp. (15–19.8 mm), *F. oxysporum* (11.5–19.77 mm), *A. alternata* (20.83 mm), *A. niger* (13–14.47 mm), and *A. flavus* (12.17–12.83 mm). Similar to that of the antibacterial activity, the marked antifungal activity of SCE could be likely due to the presence of secondary metabolites, such as tannins, terpenoids, saponins, and flavonoids. These phytochemicals are also known to possess antifungal properties, with tannins directly affecting fungi [62]. Their antifungal action is attributed to their lipophilicity, hydroxyl groups, and ability to bind proteins and adhesins, disrupting membranes, inactivating enzymes, and binding metal ions, leading to toxic effects on fungal cells [63].

Among the routinely used chemical disinfectants whose active ingredient was BC, CD1 showed good activity against most of the fungal isolates, followed by CD2. This study showed a significant difference ($p < 0.05$ according to Tukey’s HSD

Table 3. Antibacterial activity comparison of SCE and chemical disinfectants against the bacterial environmental floor isolates.

Sampling sites	Bacterial isolates	SCE	CD-1	CD-2	CD-3
Patient waiting area	<i>Bacillus</i> sp. 1	15.33 ± 0.57 ^b	20.9 ± 0.66 ^a	21.33 ± 0.58 ^a	0.0 ± 00 ^c
	<i>Bacillus</i> sp. 2	17.37 ± 0.55 ^a	18.6 ± 0.66 ^a	13.53 ± 0.61 ^b	0.0 ± 00 ^c
	<i>Staphylococcus aureus</i>	22.73 ± 1.10 ^a	12.57 ± 0.6 ^b	10.7 ± 0.96 ^b	0.0 ± 00 ^c
	<i>Pseudomonas aeruginosa</i>	14.13 ± 1.03 ^a	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	0.0 ± 00 ^b
Hospital laboratory	<i>Staphylococcus</i> sp.1	21.57 ± 0.51 ^b	24.67 ± 0.58 ^a	23.57 ± 0.55 ^a	11.4 ± 0.53 ^c
	<i>Bacillus</i> sp. 1	20.5 ± 0.5 ^b	23.77 ± 0.68 ^a	19.33 ± 0.58 ^b	0.0 ± 00 ^c
	<i>Staphylococcus</i> sp.2	18.07 ± 0.40 ^c	24.37 ± 0.55 ^a	20.90 ± 0.36 ^b	0.0 ± 00 ^d
	<i>Enterobacter hormaechei</i>	20.33 ± 1.5 ^a	0.0 ± 00 ^b	0.0 ± 00 ^b	0.0 ± 00 ^b
Hospital reception	<i>Bacillus</i> sp. 1	16.1 ± 0.66 ^b	18 ± 1 ^a	18.83 ± 0.29 ^a	0.0 ± 00 ^c
	<i>Bacillus</i> sp. 2	19.1 ± 0.26 ^b	25.17 ± 0.29 ^a	24.9 ± 0.57 ^a	14.1 ± 0.85 ^c
	<i>P. aeruginosa</i>	18.3 ± 0.75 ^a	0 ± 0 ^b	0 ± 0 ^b	0.0 ± 00 ^b
	<i>Bacillus</i> sp. 3	12.8 ± 0.34 ^b	17.63 ± 0.6 ^a	16.6 ± 0.53 ^a	0.0 ± 00 ^c
Lecture hall	<i>S. aureus</i>	18.83 ± 1.01 ^b	19.13 ± 0.32 ^{ab}	20.67 ± 0.76 ^a	0.0 ± 00 ^c
	<i>S. aureus</i>	25.3 ± 0.61 ^a	23.6 ± 1.04 ^a	21.13 ± 1.03 ^b	11.5 ± 0.5 ^c
	<i>Bacillus</i> sp.1	17.53 ± 0.5 ^a	18.23 ± 0.87 ^a	18.1 ± 0.85 ^a	0 ± 0 ^b
	<i>Bacillus</i> sp.2	19.27 ± 0.6 ^a	16 ± 1 ^b	12.4 ± 0.79 ^c	0 ± 0 ^d
Staircase	<i>Staphylococcus</i> sp.1	20.03 ± 0.45 ^b	24.17 ± 1.02 ^a	21.7 ± 0.62 ^b	0 ± 0 ^c
	<i>Bacillus</i> sp.1	15.8 ± 0.62 ^c	18.33 ± 0.56 ^b	21.43 ± 0.51 ^a	0 ± 0 ^d
	<i>Bacillus</i> sp.2	15.37 ± 0.47 ^b	19 ± 1 ^a	17.7 ± 0.61 ^a	0 ± 0 ^c
	<i>Bacillus</i> sp.3	18.5 ± 0.5 ^b	21.53 ± 0.5 ^a	19.67 ± 0.76 ^b	0 ± 0 ^c
Department of Microbiology	<i>Bacillus</i> sp.4	18.6 ± 0.66 ^b	18.67 ± 0.76 ^b	21.5 ± 0.5 ^a	0 ± 0 ^c
	<i>S. aureus</i>	22.73 ± 1.10 ^b	24.33 ± 0.58 ^{ab}	26.17 ± 1.04 ^a	0 ± 0 ^c
	<i>Bacillus</i> sp.1	16.9 ± 0.57 ^c	25.5 ± 0.56 ^a	19.63 ± 0.71 ^b	0 ± 0 ^d
	<i>Bacillus</i> sp.2	19.9 ± 0.66 ^a	20 ± 1 ^a	13.1 ± 0.85 ^b	0 ± 0 ^c
Library	<i>Bacillus</i> sp.3	15.97 ± 1.06 ^c	19.6 ± 0.06 ^b	25.4 ± 0.6 ^a	0 ± 0 ^d
	<i>Providencia rettgeri</i>	21.83 ± 1.04 ^a	0 ± 0 ^b	0 ± 0 ^b	0 ± 0 ^b
	<i>Staphylococcus</i> sp.1	18.17 ± 0.29 ^a	12.83 ± 0.76 ^b	17.1 ± 0.79 ^a	0 ± 0 ^c
	<i>Bacillus</i> sp.2	15.33 ± 1.53 ^b	21.43 ± 0.51 ^a	21.7 ± 0.62 ^a	0 ± 0 ^c
Office	<i>Bacillus</i> sp.3	15 ± 1 ^a	16.4 ± 0.53 ^a	16.33 ± 0.58 ^a	0 ± 0 ^b
	<i>Bacillus</i> sp.1	17.5 ± 0.5 ^a	17.63 ± 0.56 ^a	15.6 ± 0.53 ^b	0 ± 0 ^c
	<i>Bacillus</i> sp.2	20 ± 1 ^b	20.5 ± 0.56 ^{ab}	21.93 ± 0.6 ^a	0 ± 0 ^b
	<i>Staphylococcus</i> sp.1	18.83 ± 1.04 ^a	20.57 ± 0.98 ^a	19 ± 1 ^a	0 ± 0 ^b
Mess	<i>Staphylococcus</i> sp.1	16 ± 1 ^b	24 ± 1 ^a	15.77 ± 0.68 ^b	10.83 ± 0.76 ^c
	<i>Bacillus</i> sp.1	15.33 ± 1.26 ^c	22.4 ± 0.53 ^a	19.93 ± 0.6 ^b	0 ± 0 ^d
Corridor	<i>S. aureus</i> 1	23 ± 0.7 ^a	21.33 ± 0.58 ^b	20.17 ± 0.76 ^b	0 ± 0 ^c
	<i>Bacillus</i> sp.1	18.5 ± 0.5 ^b	20.77 ± 0.68 ^a	20.47 ± 0.81 ^a	0 ± 0 ^c
	<i>Bacillus</i> sp.2	15.33 ± 1.53 ^b	18.63 ± 0.7 ^a	16.1 ± 1.01 ^{ab}	0 ± 0 ^c
Auditorium	<i>Bacillus</i> sp.1	15.8 ± 0.98 ^b	19.6 ± 0.72 ^a	21 ± 1 ^a	0 ± 0 ^c
	<i>Bacillus</i> sp.2	15 ± 1 ^b	19.5 ± 0.87 ^a	19.43 ± 0.84 ^a	0 ± 0 ^c
	<i>S. aureus</i>	19.53 ± 0.68 ^a	18.67 ± 0.76 ^a	14.57 ± 0.6 ^b	0 ± 0 ^c
	<i>Micrococcus</i> sp.	28.87 ± 0.81 ^b	37.23 ± 0.49 ^a	38.83 ± 0.76 ^a	17.17 ± 0.76 ^c

Different letters at each row denote significantly different ($p < 0.05$) according to Tukey's HSD post hoc test.

post hoc test) between the SCE and chemical disinfectants on antifungal activity against the isolates. CD1 showed antifungal activity against all the fungal isolates except one *Mucor* sp., which was isolated from the patient waiting area. Similarly, CD2 showed antifungal activity against all fungal isolates except that one *Mucor* sp., which was isolated from the patient waiting area.

However, CD3 does not show activity in all the fungal isolates. Stupar *et al.* [64] revealed that BC showed antifungal activity against *A. niger*, *Bipolaris spicifera*, *Penicillium* sp., *A. ochraceus*, *Epicoccum nigrum*, and *Trichoderma viride*, which were isolated from cultural heritage objects. BC showed antifungal activity against *Aspergillus* sp. and *Fusarium* sp. [65]. It is noteworthy

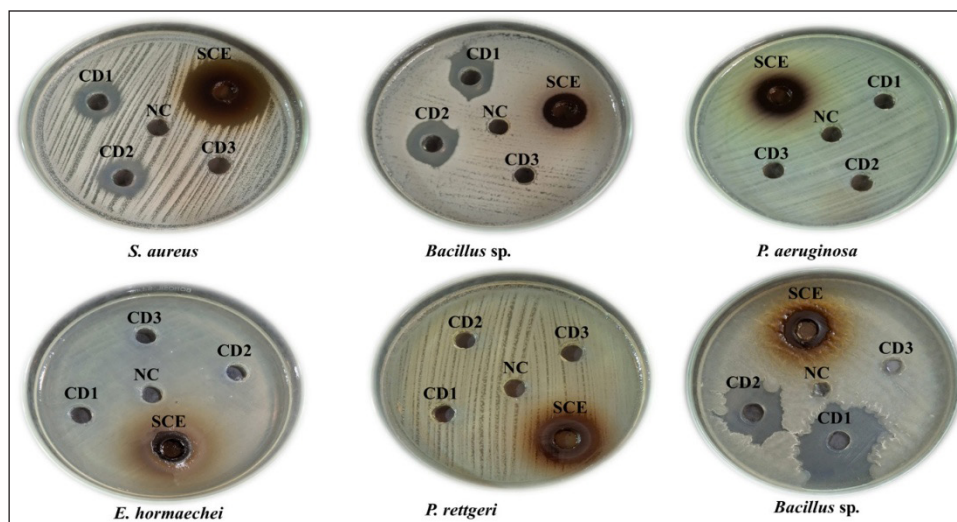


Figure 4. Zone of inhibition of SCE and chemical disinfectants against the bacterial environmental floor isolates.

Table 4. Antifungal activity comparison of SCE and chemical disinfectants against the fungal environmental floor isolates.

Sampling sites	Fungal isolates	SCE	CD-1	CD-2	CD-3
Patient waiting area	<i>Mucor</i> sp.	23 ± 1.41 ^a	0 ± 0 ^b	0 ± 0 ^b	0 ± 0 ^b
	<i>Fusarium</i> sp.	11.5 ± 0.41 ^b	14.97 ± 1 ^a	11.6 ± 0.66 ^b	0 ± 0 ^c
Hospital laboratory	<i>Penicillium</i> sp.	15.5 ± 0.5 ^b	14.83 ± 0.74 ^b	21.7 ± 0.7 ^a	0 ± 0 ^c
	<i>Aspergillus niger</i>	13 ± 1 ^b	23.5 ± 1.32 ^a	21 ± 1.32 ^a	0 ± 0 ^c
	<i>Aspergillus flavus</i>	12.17 ± 0.76 ^b	15 ± 1 ^a	12.87 ± 0.81 ^a	0 ± 0 ^c
Reception	<i>A. flavus</i>	12.3 ± 1.13 ^b	14.33 ± 0.58 ^a	12.7 ± 0.26 ^{ab}	0 ± 0 ^c
	<i>Alternaria alternata</i>	20.83 ± 1.04 ^a	11.73 ± 0.25 ^b	21.4 ± 1.04 ^a	0 ± 0 ^c
Lecture hall	<i>A. flavus</i>	12.83 ± 0.21 ^b	14.93 ± 0.9 ^a	12.77 ± 0.68 ^b	0 ± 0 ^c
Staircase	<i>Penicillium</i> sp.	15.8 ± 0.72 ^b	13.5 ± 0.62 ^c	21.43 ± 0.55 ^a	0 ± 0 ^d
	<i>Fusarium</i> sp.	19.77 ± 0.68 ^a	21.17 ± 1.26 ^a	12.87 ± 0.81 ^b	0 ± 0 ^c
Department of Microbiology	<i>A. niger</i>	13.33 ± 1.15 ^b	23.67 ± 1.53 ^a	20.6 ± 1.51 ^a	0 ± 0 ^c
	<i>Penicillium</i> sp.	19.8 ± 0.72 ^a	18.07 ± 0.9 ^b	13.6 ± 0.6 ^c	0 ± 0 ^d
Library	<i>A. niger</i>	14.47 ± 0.57 ^c	24 ± 1 ^a	21.97 ± 1.05 ^b	0 ± 0 ^d
	<i>Penicillium</i> sp.	15 ± 1 ^b	12.93 ± 0.9 ^c	21.4 ± 0.53 ^a	0 ± 0 ^d
Office	<i>Mucor</i> sp.	26.33 ± 2.1 ^a	13.67 ± 1.15 ^b	10.7 ± 1.54 ^b	0 ± 0 ^c
Mess	<i>Penicillium</i> sp.	18.7 ± 1.47 ^b	26.67 ± 1.53 ^a	25.97 ± 0.45 ^a	0 ± 0 ^c
Corridor	<i>A. niger</i>	13.67 ± 0.58 ^c	24.93 ± 0.9 ^a	21.8 ± 0.35 ^b	0 ± 0 ^d
	<i>A. niger</i>	11.7 ± 0.62 ^b	16.13 ± 0.81 ^a	10.87 ± 0.81 ^b	0 ± 0 ^c
Auditorium	<i>Fusarium oxysporum complex</i>	13.43 ± 0.6 ^c	24.07 ± 0.9 ^a	21.9 ± 0.95 ^b	0 ± 0 ^c
	<i>A. niger</i>	13.1 ± 1.01 ^c	24.37 ± 0.55 ^a	20.03 ± 0.85 ^b	0 ± 0 ^d

Different letters at each row denotes significantly different ($p < 0.05$) according to Tukey's HSD post hoc test.

that this is the first study to report on the resistance of *Mucor* sp. isolated from environmental floors to BC.

Antibiotic drug susceptibility

Antibiotic drug susceptibility test was done by the disc diffusion method against the environmental Gram-positive and Gram-negative bacterial isolates. Results were interpreted according to the CLSI guidelines [52]. In the case of Gram-positive isolates, it showed resistant of 32.4% (12/37) to B, 27% (10/37)

to AMX, 24.3% (9/37) to E, 21.6% (8/37) to CEP and NV, 10.8% (4/37) to O, and it showed 100% (35/35) sensitive to AK and V (Fig. 6). Among the Gram-positive isolates, *Staphylococcus* sp. showed resistant of 58.33% (7/12) to AMX, 50% (6/12) to E, 41.67% (5/12) to NV, and 100% (12/12) sensitive to B, CEP, O, V, and AK. *Bacillus* sp. showed 52% (12/24) resistant to B, 33.33% (8/24) to CEP, 16.67% (4/24) to O, 12.5% (3/24) to AMX, E, and NV, and exhibited 100% 9 (24/24) sensitive to V and AK. *Micrococcus* sp. showed 100% (1/1) sensitivity to

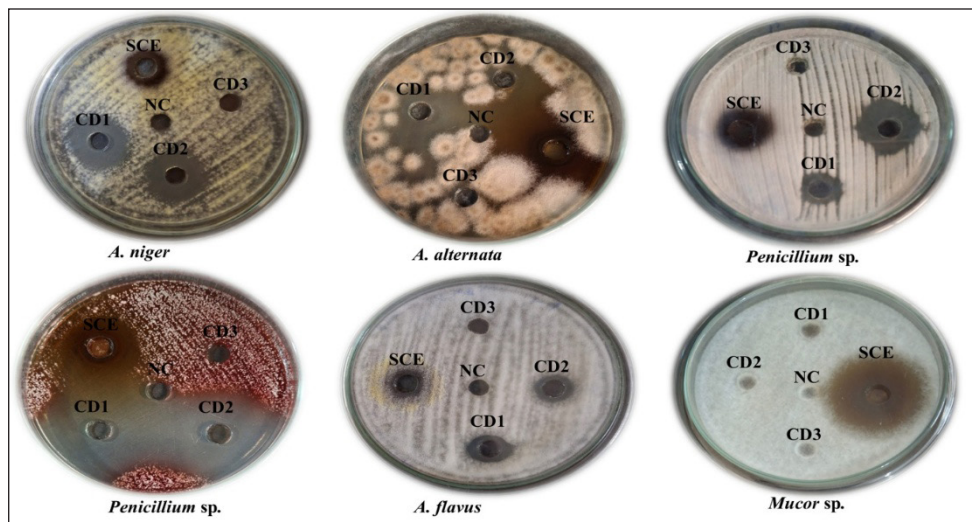


Figure 5. Zone of inhibition of SCE and chemical disinfectants against the fungal environmental floor isolates.

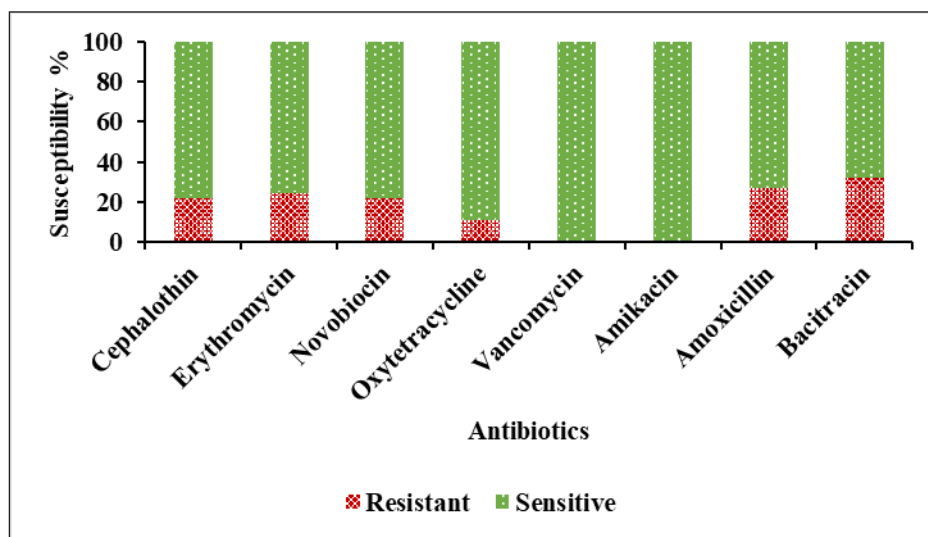


Figure 6. Percentage of antibiotic susceptibility against the Gram-positive environmental isolates.

all the tested antibiotics. These findings were consistent with the previous study by Mohammed *et al.* [66] who reported that *Staphylococcus* sp. isolated from hospital environment showed 67% resistant to E, and 21% to V, and *Bacillus* sp. showed 8% resistant to E and sensitive to V. *B. cereus* strains isolated from fresh vegetables were 99% resistant to amoxicillin/clavulanic acid combination and 4.5% to E [67]. *Micrococcus* sp. showed 100% (1/1) sensitivity to all the tested antibiotics. This is the first study to report the antibiotic susceptibility pattern of *Micrococcus* sp. isolated from environmental floors.

In the case of Gram-negative isolates, it showed resistant of 100% (4/4) to NIT, 50% (2/4) to K, 50% (2/4) to TE, 25% (1/4) to S and CM, and it showed 100% sensitive to CIP, AK and CB. (Fig. 7). Among the Gram-negative isolates, *P. aeruginosa* showed resistant of 100% (2/2) to NIT and K, 50% (1/2) to CM and TE, and 100% (2/2) sensitive to CIP, S, AK and CB. Eyo *et al.* [68] reported that environmental isolates of *P.*

aeruginosa showed resistant to CB, CIP, and AK. Low resistant to AK and CIP against *P. aeruginosa* isolated from the hospital environmental isolates [69]. *Pseudomonas aeruginosa* isolated from residential sewage was resistant to NIT and CIP [70]. Morita *et al.* [71] reported that *P. aeruginosa* was resistant to TE. *Enterobacter hormaechei* showed resistant of 100% (1/1) to NIT and showed 100% (1/1) sensitive to CIP, K, CM, TE, S, AK, and CB. The result was in accordance with the previous finding [72]. *Providencia rettgeri* showed resistant of 100% (1/1) to NIT and TE and showed 100% (1/1) sensitive to CIP, K, S, CM, AK, and CB which was supported by the previous study [73].

Antifungal drug susceptibility

Antifungal drug susceptibility test was investigated by the disc diffusion method against the environmental fungal isolates. The results were interpreted based on CLSI

guidelines [74]. They showed resistant of 100% (20/20) to FLC, 30% (6/20) to Itraconazole (ITR), 15% (3/20) to AMP, and 10% (1/20) to CC (Fig. 8). However, all the fungal isolates were 100% (20/20) sensitive to NS and KT. The results are in accordance with the previous study [75]. Among the fungal isolates, *Mucor* sp. showed 100% (2/2) resistant to FLC and 50% (1/2) to AMP and ITR and showed 100% (2/2) sensitive to NS, CC, and KT. *Alternaria alternata* showed resistant of 100% (1/1) to FLC, ITR, and CC and showed 100% (1/1) sensitive to NS, AP, and KT. *Penicillium* sp. showed resistant of 100% (5/5) to FLC and 20% (1/5) to ITR and produced 100% (5/5) sensitive to NS, AP, CC, and KT. *Fusarium oxysporum* showed 100% resistant to FLC, 33.33% (1/3) to ITR and CC, and 100% (3/3) sensitive to NS, AP, and KT. Moreover, it showed 66.66% (2/3) sensitive to ITR and CC.

Aspergillus niger showed 100% (8/8) resistant to FLC, 16.67% (1/6) to AMP and ITR, and 100% sensitive to NS, CC, and KT. *Aspergillus flavus* showed 100% resistant to FLC, 33.33% (1/3) to AMP and ITR, and 100% sensitive to NS, CC, and KT. Consistent with our findings, the environmental fungal isolates such as *Aspergillus* sp. and *Mucor* sp. were highly sensitive to AMP but had low susceptibility against *Fusarium* sp. [76] However, *Aspergillus* sp. *Mucor* sp. and *Fusarium* sp. had low susceptibility toward ITR. *Penicillium* sp. and *Alternaria* sp. were highly sensitive to AMP and ITR. Kaur et al. [77] revealed that *A. flavus* and *A. niger* isolated from hospital environments as well as from community environments have low MIC for ITR and high MIC for AMP. *Aspergillus niger*, *A. flavus*, *Penicillium* sp. And *Mucor* sp. isolated from poultry environments were sensitive to NS but resistant to FLC [78].

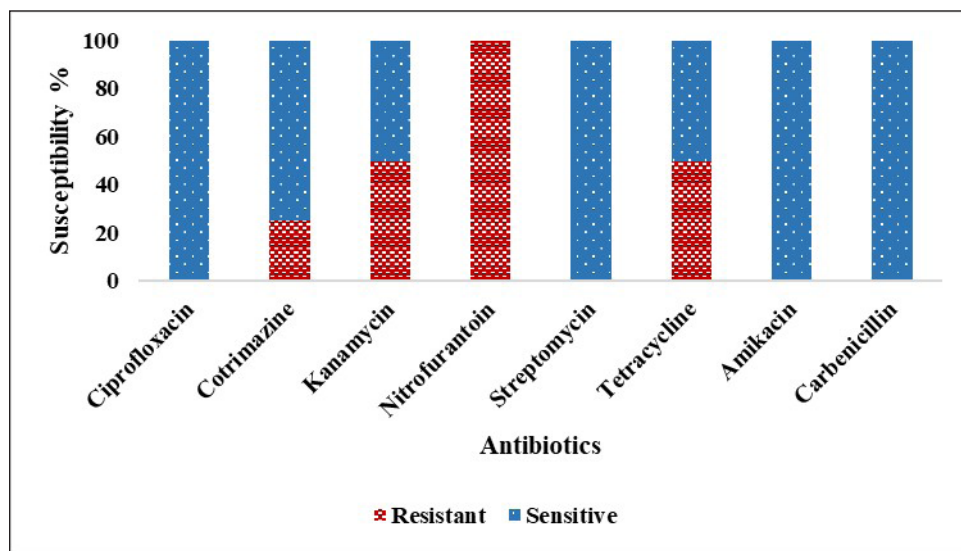


Figure 7. Percentage of antibiotic susceptibility against the Gram-negative environmental isolates.

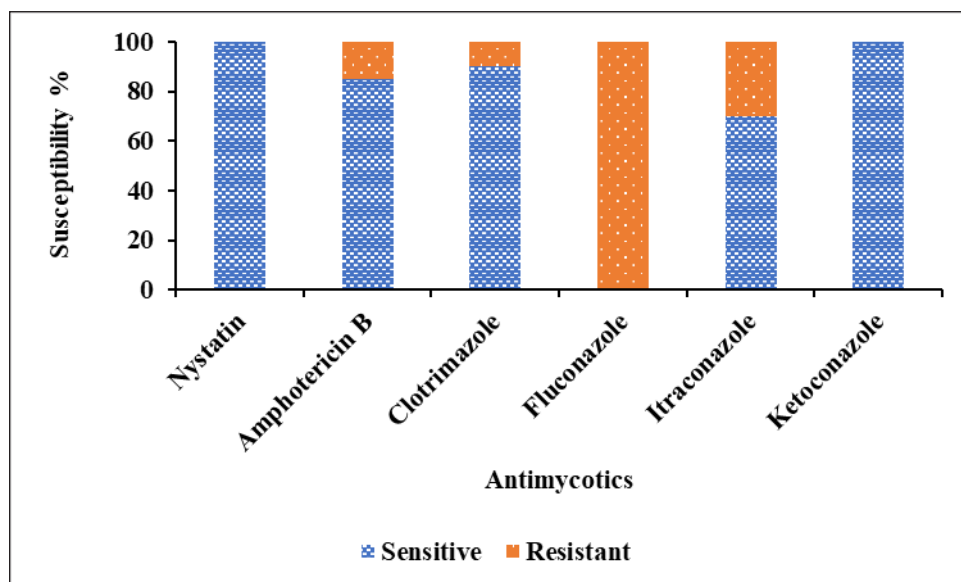


Figure 8. Percentage of antifungal susceptibility against the fungal environmental isolates.

CONCLUSION

Syzygium cumini leaf extract showed significant antimicrobial activity against all the bacterial and fungal environmental floor isolates. *Syzygium cumini* extract showed a significant antimicrobial activity against the chemical disinfectant-resistant and antibiotic-resistant environmental isolates, supporting the potential use of *S. cumini* extract as an alternative plant-derived floor disinfectant that is eco-friendly and cost-effective. Identification of disinfectant-resistant genes should be done from the disinfectant-resistant isolates for future studies.

AUTHORS' CONTRIBUTION

All the authors made significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICS STATEMENT

Ethical approvals details are given in the 'Material and Method' Section.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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