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Investigation of the antimicrobial efficacy and cytotoxicity of a natural disinfectant *Syzygium cumini* (L.) skeels leaf extract on vero cell lines

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ABSTRACT

Disinfectants are vital for infection prevention and environmental health maintenance. However, the use of chemical disinfectants has adverse effects on human health, including the development of antibiotic-resistance genes and resistance to disinfectants due to overuse and abuse. There is a critical need to find natural alternatives to reduce the problems caused by chemical disinfectants. The present study analyzed the antimicrobial activity of *Syzygium cumini* leaf extract (SCLE) against *Staphylococcus aureus, Pseudomonas aeruginosa, Mucor* sp., *Candida albicans*, and *Aspergillus niger* and its efficacy as a natural disinfectant was also evaluated, along with GC-MS analysis and *in vitro* toxicity test. The study showed that SCLE exhibited antimicrobial activity against all test organisms except *A. niger*. The minimum inhibitory concentration (MIC) against *S. aureus, P. aeruginosa, Mucor sp*., and *C. albicans* was 625, 1,250, 78, and 1,250 µg/ml, respectively. The minimum bactericidal concentration (MBC) against *S. aureus* and *P. aeruginosa* was 1,250 and 2,500 µg/ml, respectively. The minimum fungicidal concentration (MFC) against Mucor sp. and C. albicans was 156 and 5,000 µg/ml, respectively. SCLE demonstrated bactericidal and fungicidal properties based on MIC: MBC and MIC: MFC, with significant reductions in *P. aeruginosa* (99.99%), *S. aureus* (90.71%), *Mucor* sp. (92%), and *C. albicans* (73.45%) after the treatment. *Syzygium cumini* leaf extract, identified as nontoxic with an IC50 of 320µg/ml, proves promising as a potent natural disinfectant. This study marks the first report on the efficacy of *S. cumini* leaf extract against test organisms, showcasing its potential as a natural disinfecting agent against test organisms.

INTRODUCTION

Microorganisms are ubiquitous, both inside and outside the human body, eventually settling on surrounding surfaces, including floors. Most common floor microflora are opportunistic and can cause infections at higher concentrations [1]. *Staphylococcus aureus*, a ubiquitous and pathogenic organism to humans, triggers various skin infections such as carbuncles, impetigo, cellulitis, furuncles, and so on. It can also lead to pneumonia, endocarditis, osteomyelitis, sepsis, and so on. [2]. *Pseudomonas aeruginosa*, an opportunistic human pathogen, causes diseases such as generalized inflammation and sepsis [3] *Candida albicans* can survive on hospital surfaces and fomites, causing opportunistic infections like oral thrush, vaginal infection, and so on [4,5]. *Aspergillus niger*, an opportunistic pathogen, can lead to allergic bronchopulmonary aspergillosis or invasive aspergillosis [6]. Mucor mold, an opportunistic fungus, causes mucormycosis [7]. Due to the COVID-19 pandemic in India, mucormycosis has emerged as a serious complication during its second wave [8]. Disinfectants

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play a significant role in preventing infections and maintaining environmental health [9]. The usage of chemical disinfectants has significantly increased in hospitals, laboratories, and households due to the earlier COVID-19 pandemic. However, it causes various side effects, such as irritation to the respiratory system, skin, and eyes. Cases of poisoning due to excessive usage and reports of disinfectant adulteration have also been documented [10]. Overuse and abuse of chemical disinfectants lead to antibiotic resistance genes and resistance to disinfectants [9]. Therefore, finding a natural disinfectant is crucial to reducing the problems caused by chemical disinfectants. A natural disinfectant should be easy to use, noncorrosive, harmless on most surfaces, safe on the skin and for breathing, and eco-friendly [11]. Studies were carried out effectiveness of different plant extracts such as neem leaves [12,13], turmeric [14], aloe vera, and gotu kola [15] as a natural disinfectant. However, ascertaining the cytotoxic nature of herbal plants is essential, which is why the MTT test is frequently employed to assess the viability of cells at plant concentrations [16].

There is a demand for an effective natural disinfectant which is of societal demand for safer alternatives to chemical disinfectants, that are known to have significant side effects. *Syzygium cumini* (Jamun) is a large evergreen tree of the Myrtaceae family with various medicinal properties [17,18]. Its leaf extract has antibacterial [17,19], antifungal [17], antidiabetic activity [20]. To the best of our knowledge, the effectiveness of *S. cumini* leaf extract as a disinfecting agent was not investigated. Therefore, evaluating the antibacterial and antifungal activity of *Syzygium cumini* leaf extract (SCLE), determining the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) of the extract, calculating MBC/MIC and MFC/MIC ratios, and assessing the effectiveness of SCLE as a disinfecting agent were the focuses of this study. Additionally, Gas Chromatography-Mass Spectroscopy (GC-MS) analysis and *in vitro* toxicity tests of SCLE by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay were conducted.

MATERIALS AND METHODS

Collection and preparation of extract

Syzygium cumini (L.) Skeel leaves were collected from Perumbakkam, Chennai, Tamil Nadu, and authenticated by Dr. P. Palani, Centre for Advanced Studies in Botany, University of Madras, Chennai, Tamil Nadu. *Syzygium cumini* extract was prepared according to Elfadil *et al.* [17] with slight modification. The collected leaves were washed properly, shade dried, powdered, and used for the extraction with distilled water (1:10). It was heated at 60°C for 2 hours. Then, it was filtered, dried, and used for the study.

Cultures

Bacterial cultures such as *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 29213) were obtained from Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu. Fungal cultures such as *C. albicans*, *Mucor* sp., and *A. niger* were obtained from the Department of Microbiology, University of Madras, Chennai, Tamil Nadu.

Antibacterial and antifungal activity

The antibacterial and antifungal activity of SCLE was evaluated by the agar well diffusion method [21]. Muller Hinton agar plates were inoculated with the test organisms such *P. aeruginosa, S. aureus, Mucor* sp., *C. albicans.,* and *A. niger*. A stock solution of 500,000 µg/ml concentration of SCLE was prepared. From the stock solution, 100 µl was loaded into the wells. Ciprofloxacin and Nystatin were used as a positive control for the tested bacterial and fungal cultures, respectively. As a negative control, distilled water was used. The diameter of the inhibition zone was measured after incubation at 37°C for 1 day for bacterial cultures and for 3 days at room temperature (RT) for fungal cultures, respectively. The assay was conducted in triplicates.

MIC

MIC of SCLE was determined by the agar well diffusion method [22]. Different concentrations of SCLE (µg/ml) such as 38, 78, 156, 312.5, 625, 1,250, 2,500, 5,000, and 10,000 were prepared. Lawn cultures of the used organisms were made onto the sterile Muller Hinton agar plates. 100 µl of different concentrations were loaded into the respective wells. The plates were then incubated.

MBC and MFC

Streaks were taken from the inhibition zone of MIC plates and inoculated onto respective nutrient agar (NA) and Sabouraud Dextrose agar (SDA) plates and then incubated. The concentration of the extract which did not show microbial growth on the respective agar plates was determined [23].

Then, the MBC/MIC and MFC/MIC ratio of SCLE against the test organisms were calculated to determine if the extract has bactericidal/fungicidal (MBC/MIC≤4 or MFC/MIC≤4) or bacteriostatic/fungistatic (MBC/MIC>4 or MFC/MIC>4) activity [24,25].

Efficacy of SCLE as a disinfecting agent

5%, 10%, and 50% concentrations of the plant extract were prepared to evaluate its effectiveness as a disinfecting agent. The procedure was followed according to Welk *et al.* [26] with some modifications. 10 µl of bacterial (1×10^5CFU/ml) or fungal suspension (1×10^4CFU/ml) was added separately to 0.99 ml of each concentration at RT, respectively. The control contained 0.99 ml of distilled water instead of extract. After 15 and 30 minutes of contact time, it was serially diluted and plated onto NA and SDA plates which were then incubated at 37°C for 24 hours for bacterial cultures and 48 hours at RT for fungal cultures, respectively. After incubation, colonies were counted.

The percentage decrease in microorganism counts following treatment with varying concentrations of extract for 15 minutes and 30 minutes was calculated [27].

GC-MS analysis

GCMS-QP 2010 Ultra (Shimadzu, Japan) was used to detect the compounds present in the extract. Their mass

spectrum was compared with the reference spectrum available in the Willey 8 and NIST 11 and 17 libraries.

In vitro toxicity test

The *in vitro* cytotoxicity test of *S. cumini* leaves was evaluated using Vero cell lines by MTT assay [28]. The cells were seeded in Dulbecco's modified eagle medium (100 µl) including 10% fetal bovine serum, streptomycin, penicillin, gentamycin, amphotericin B, and L-glutamine in a 96-well plate at 1.0×10^4 cells/ml and incubated at 37°C for 1 day. 1–1,000 µg/ml concentrations of SCLE were used for cytotoxicity. After incubation with the extract, the MTT reagent was added into each well and incubated for 4 hours. Then, dimethyl sulphoxide was added after removing the supernatant. The absorbance was then measured at 580 nm. The cell viability (%) was calculated according to Fouda *et al.* [29].

STATISTICAL ANALYSIS

One-way ANOVA and post hoc Duncan test were used to determine the significance level ($p < 0.05$) of data (IBM SPSS statistics version 25.0).

RESULTS AND DISCUSSION

Antibacterial and antifungal activity of extract

SCLE demonstrated antimicrobial activity against *S. aureus*, *P. aeruginosa, C. albicans, Mucor* sp., and *A.niger.* The results of antibacterial and antifungal activity are shown in Figure 1. Table 1 displays the zone of inhibition (mm) against the test organisms. SCLE inhibited all the tested microbial cultures with an inhibition zone range from 20–35 mm but was not effective on *A. niger.* Elfadil *et al.* [17] revealed that *S. cumini* water extract has antimicrobial activity against *S. aureus* and *C. albicans* but none against *Pseudomonas* sp. and *A. niger*. Pareek *et al.* [30] reported that the aqueous extract of *S. cumini* exhibits antifungal activity against *C. albicans* and *A. niger*. The difference in the results might be due to the difference in the extraction process. Consistent with our findings, SCLE has antifungal activity against *C. albicans*. [31].

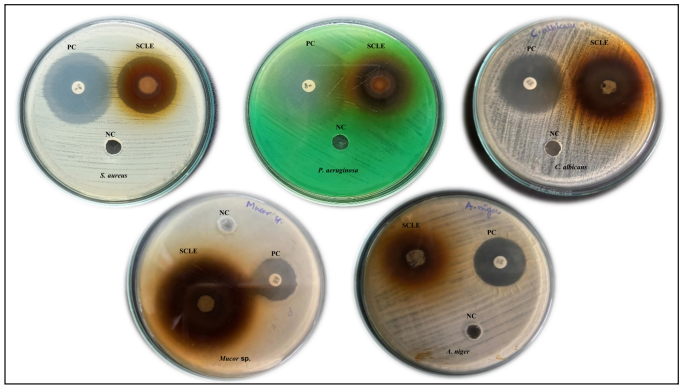

Figure 1. Antibacterial and antifungal activity of SCLE.

PC, Positive control; NC, Negative control; SCLE, *S. cumini* leaf extract.

Table 1. Zone of inhibition (mm) of SCLE against test organisms and data were expressed as mean ± SD.

Organisms	SCLE	Positive control
S. aureus	26.17 ± 0.76	24.06 ± 0.8
P. aeruginosa	20 ± 0.89	29.9 ± 1
C. albicans	25.01 ± 0.63	23 ± 0.3
Mucor sp.	35.04 ± 0.35	21 ± 0.2
A. niger	-	22 ± 0.5

MIC

The inhibitory concentration of SCLE against test organisms is illustrated in Figure 2. The MIC value of SCLE ranged from 0.078–1.25 µg/ml. MIC of SCLE against *S. aureus*, *P. aeruginosa*, *Mucor* sp.*, and C. albicans* was found to be 625 µg/ml, 1,250 µg/ml, 78 µg/ml, and 1,250 µg/ml, respectively. The lowest inhibitory concentration was found against *Mucor* sp. A previous study reported that *S. cumini* aqueous extract had MIC of 1,560 µg/ml against *C. albicans* [31]. Chanudom *et al.* [32] found that the MIC of the aqueous extract of *S. cumini* against *S. aureus* was 6.25 mg/ml. Oliveira *et al.* [33] reported MIC of *S. cumini* hydroalcoholic extract against *S. aureus*, *P. aeruginosa*, and *C. albicans* as 20 µg /ml, 90 µg/ml, and 90 µg /ml, respectively]. Furthermore, hydroalcoholic extract of *S. cumini* leaves has 1,296.8µg/ml against S. *aureus*. The variation in the values might be due to the difference in extraction process and concentration tested.

MBC

The MBC of SCLE against *S. aureus* and *P. aeruginosa* was 1,250 µg/ml and 2,500 µg/ml, respectively. Chanudom *et al.* [32] reported an MBC of the aqueous extract of *S. cumini* against *S. aureus* as 12.5 mg/ml. In this study, the MBC/MIC ratio for SCLE was determined to evaluate its bactericidal or bacteriostatic activity. SCLE. Its MBC/MIC against *S. aureus* and *P. aeruginosa* was 2, indicating bactericidal activity which could be due to its bioactive constituents. Consistent with our finding, hydroalcoholic extract of *S. cumini* leaves had a bactericidal effect against *S. aureus* [34].

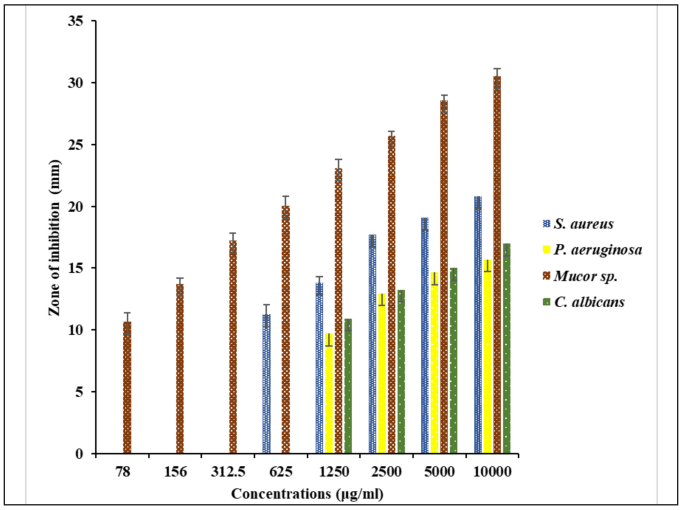

Figure 2. MIC of SCLE against test organisms.

MFC

The MFC of SCLE against *C. albicans and Mucor* sp. was 5,000 µg/ml and 156 µg/ml, respectively. The MFC/MIC ratio for SCLE was further analyzed to evaluate its fungicidal or fungistatic activity. The MFC/MIC ratio of SCLE against *C. albicans and Mucor* sp. was 4 and 2, signifying fungicidal activity. This fungicidal activity against all tested fungi could be due to the bioactive constituents in SCLE. Figueirêdo Junior *et al.* [35] reported that the hydroalcoholic extract of *S. cumini* leaves had a fungistatic effect against *C. albicans* [35].

Efficacy of SCLE as a disinfecting agent

The effectiveness of SCLE as a disinfecting agent against bacterial and fungal cultures is illustrated in Figure 3. SCLE was effective against *P. aeruginosa* and *S. aureus* within the tested contact times. Against fungi, SCLE was also effective against *Mucor sp.* and *C. albicans*. Reduction percentages after treatment with SCLE are detailed in Table 2.

In this study, tested bacterial and fungal cultures were significantly reduced after treatment with SCLE. The disinfectant effectiveness of SCLE against the tested organisms was arranged as (*P. aeruginosa* > *S. aureus*> *Mucor* sp.> *C. albicans*). SCLE exhibited higher disinfectant effectiveness on bacterial cultures than on fungal cultures. Among the tested concentrations, 50% SCLE with a 30-minute contact time showed the most significant reduction in the tested organisms (99.99% reduction in *P. aeruginosa*, 90.71% in *S. aureus*, 92% in *Mucor* sp., and 73.45% in *C. albicans*). This study represents the first report evaluating the efficacy of *S. cumini* leaf extract as a disinfecting agent against test organisms. Previous studies reported disinfectant effectiveness of different plant extracts [12–15]. Hidayati *et al.* [12] found that 50%, 75%, and 100% concentration of neem leaves reduced the bacteria in poultry incubators by 56.16%, 57.94%, and 62.20, respectively, and fungi in poultry incubators by 57.63%, 58.11, and 57.65%, respectively [12]. The same author also revealed that neem leaf

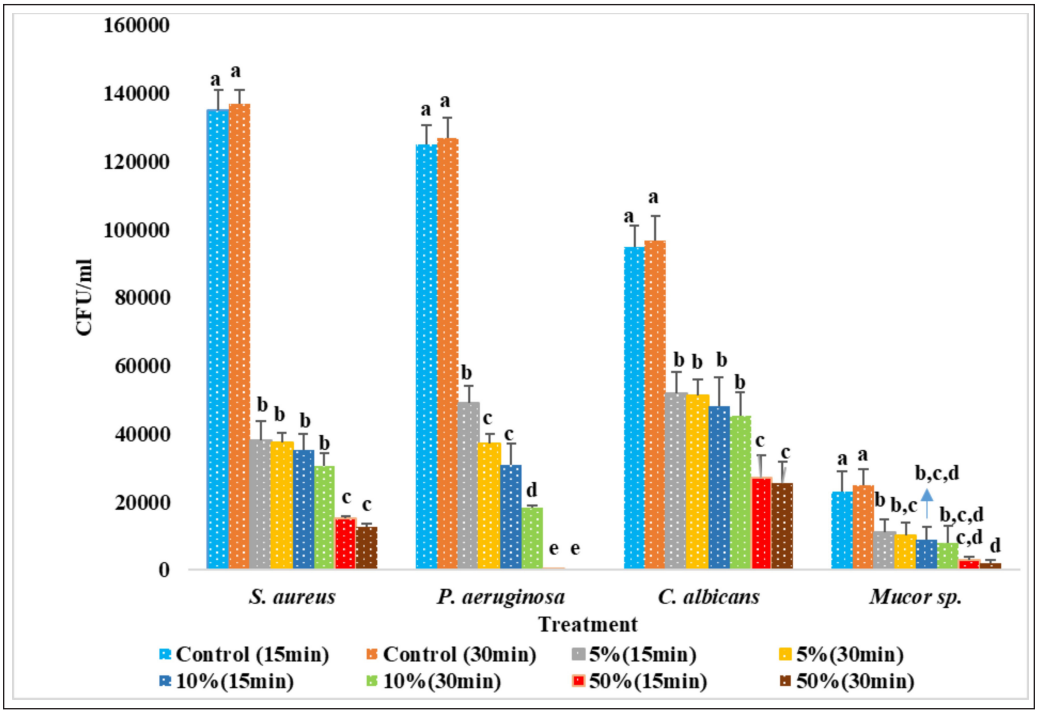

Figure 3. Efficacy of SCLE as a disinfecting agent against test organisms. $N = 3$. Mean ± SD. Different letters at same organism denotes significantly different ($p < 0.05$), according to Duncan's test.

Table 2. Reduction percentage of microorganisms after treated with SCLE.

Organisms	5% SCLE		10% SCLE		50% SCLE	
	15 minutes	30 minutes	15 minutes	30 minutes	15 minutes	30 minutes
S. aureus	71.85%	72.75%	73.83%	77.62%	88.81%	90.71%
P. aeruginosa	60.80%	70.87%	75.20%	85.59%	99.97%	99.99%
Mucor sp.	52.17%	60.00%	60.87%	68.00%	86.96%	92.00%
C. albicans	45.26%	46.89%	49.47%	53.10%	71.58%	73.45%

extract decreased the *Mucor* sp. by 50.75% [12]. Rios *et al.* [13] reported that 5% concentrations of neem leaves inhibited *S. aureus* by 99.96% at 5 minutes of contact time. Mayefis *et al.* [15] reported that 25%, 50%, and 100% concentration of aloe vera reduced 44.6%, 89.2%, and 95.3% of germs on dinner plates, respectively, and also reported that a combination of gotu kola and aloe vera at 50% and 100% concentration reduced 73.3% and 93% of the germs [15].

GC-MS analysis

Below Figure 4 displays the GC-MS chromatogram for SCLE, identifying 16 compounds presented in Table 3. Thymine has bactericidal activity against gram-negative pathogens [36]. 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- has antimicrobial activity [37] Cyclohexane 1,3,5-triphenyl- has industrial applications as a surfactant and emulsifier [38] 1-Hexacosanol possess acetylcholinesterase inhibitory activity [39]. Gamma-sitosterol has antidiabetic hypolipidemic, anticancer, antibacterial, and antiviral activity [21]. 2,2'Benzylidenebis(3-methylbenzofuran) has been reported for its anti-diabetic property [40] 5,5'-Dithiobis-(2-nitrobenzoic acid) has antiviral property [41].

In vitro cytotoxicity test

In Figure 5, the percentage of cell viability is illustrated. SCLE exhibited nontoxicity on Vero cell lines with an IC_{50} value of 320 µg/ml. Masfra and Hafni [42] reported that all extracts were considered nontoxic if the IC_{50} value was >30 µg/ml [42]. Ribeiro *et al.* [43] demonstrated low toxicity was showed in rodent macrophages, showing an IC_{50} value of 31.64 µg/ml for the hexane extract of *S. cumini* leaves. Pereira *et al.* [44] found that concentrations ranging from 10 to 200 µg/ml of *S. cumini* exhibited no toxicity on macrophage cells.

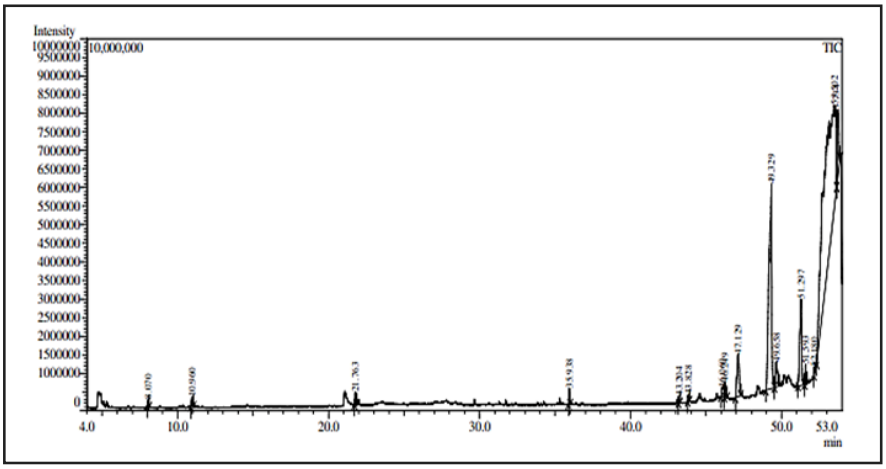

Figure 4. GC-MS chromatogram for SCLE.

Table 3. GC-MS spectral analysis of SCLE.

Peak	R. Time	Area%	Name	Formula
1	8.070	0.23	Thymine	C5H6N202
2	10.960	0.33	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4
3	21.763	0.33	Trimethylene borate	C9H18B2O6
4	35.938	0.32	Benzenepropanoic acid, 3,5-bis(1,1-dimethyl)-4-hydroxy-,ethyl ester	C19H30O3
5	43.204	0.16	DL-2-Aminoadipic acid, N-dimethylaminomethylene-,diethyl ester	C13H24N2O4
6	43.828	0.15	Cyclohexane 1,3,5-triphenyl-	C24H24
7	46.060	0.78	Peri-Xanthenoxanthene-4,10-Dione,2,8-Bis(1-Methylethyl)-	C26H20O4
8	46.249	0.73	1-Hexacosanol	C26H54O
9	47.129	3.15	8H-Dinaphtho[2,3-C:2',3'-H]Phenothiazin-8-Yl	C28H16NS
10	49.329	20.65	Furo[3'4':6,7]Naphtho [2,3-d]-1,3-dioxol-6(5aH)-one, 5,8,8a,9-tetrahydro-5-(3,4,5-trimethoxyphenyl)-,[5.alpha.,5a.beta.,alpha.)]-	C22H22O7
11	49.658	1.74	Gamma-sitosterol	C29H50O
12	51.297	5.84	4-(3-Methyl-11-Oxo-7,8,9,10,11,12-Hexahydro-benzo[B][4,7] Phenanthrolin-12-Yl)-Benzoic acid Methyl ester	C25H22N2O3
13	51.593	0.56	2,2'Benzylidenebis(3-methylbenzofuran)	C25H20O2
14	52.180	0.21	3,4-Bis(Dimethylamino)-3-Cyclobutene-1,2-Dione	C8H12N2O2
15	53.502	60.53	2-Chloro-N-[4'(2-chloro-acetylamino)-3'3-	C18H18C12N2O4
16	53.734	4.28	5,5'-Dithiobis-(2-nitrobenzoic acid)	C14H8N2O8S2

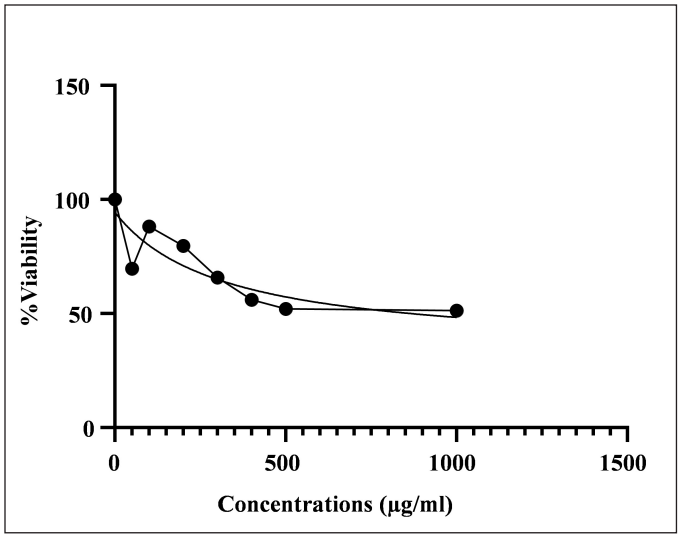

Figure 5. Percentage of viability of SCLE on vero cell lines.

Isolation of bioactive compounds from the crude extract of SCLE should be done for future studies.

CONCLUSION

Syzygium cumini leaf extract showed a bactericidal effect against *P. aeruginosa* and *S. aureus* as well as a fungicidal effect against *Mucor* sp. and *C. albicans*. Additionally, it demonstrated a notable decrease in microorganisms following SCLE treatment. The bactericidal and fungicidal activities of SCLE may be attributed to its bioactive components. It was found to be nontoxic on Vero cell lines. Thus, SCLE could be used as a potent natural disinfecting agent.

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AUTHOR CONTRIBUTIONS

All the authors made significant contributions to 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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The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

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

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