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Original Research Article

Bioactive Potential of *Cephalosporium sp.* a Fungal Endophyte Isolated from *Phyllanthus niruri* L.

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ABSTRACT

Fungal endophytes are fungi that reside within plant tissues without causing apparent harm to their hosts. These fungi produce various bioactive compounds that have potential applications in medicine. This study aimed to cultivate a *Cephalosporium* sp. fungal endophyte isolated from *Phyllanthus niruri* L., and evaluate its antimicrobial, antioxidant, and cytotoxic activities in the endophytic fungal extract. The endophyte was extracted, fractionated, and assessed for total phenolic content, antimicrobial, and antioxidant activities, as well as potential as an anti-breast cancer agent against 4T1 cell lines. Results showed that the dichloromethane fraction effectively inhibited the growth of *Salmonella typhi* with an IC_{50} value of 12.39 µg/mL. In contrast, the methanol fraction exhibited high antioxidant activity with a free radical scavenging ability of 86%. Moreover, the dichloromethane fraction demonstrated the ability to inhibit 4T1 cell growth with an IC_{50} value of 18.12 µg/ml. These findings suggest that the dichloromethane fraction has the potential as an antimicrobial and cytotoxic agent against breast cancer cells.

Keywords: Endophyte; *Phyllanthus niruri*; *Cephalosporium* sp.; Breast cancer

Introduction

In a symbiotic relationship, endophytes are small microorganisms that inhabit various parts of plants, including the xylem, phloem, roots, leaves, and fruits.[1] Among them, fungal endophytes have been recognized for producing biologically active compounds, such as alkaloids, terpenoids, and phenols, which exhibit a range of activities, including antioxidant, anticancer, antibacterial, antiviral, antifungal, and antileishmanial properties.[2,3] *Phyllanthus amarus* Schum. & Thonn. has been identified as a host plant for a diverse range of endophytic fungi and bacteria which live within its cells.[4] The presence of up to 10 different fungal endophytes in this herb has made it a valuable source for drug discovery, owing to its potential for generating compounds with various biological activities.[5]

Phyllanthus niruri Linn. belongs to the Phyllanthaceae family and offers a promising prospect for producing a fungal endophyte. This herb contains various chemical compounds, such as glycosides, alkaloids, lignin, terpenes, phenylpropanoids, flavonoids, and polyphenols.[6] Although the herb's pharmacological activities have been explored in treating bladder infections, acting as an immunomodulator, anti-inflammatory, and antiviral agent.[7] no research has been conducted on this plant species as a source of endophytes. Therefore, a comprehensive study of the extraction and biological evaluation of the fungal endophyte derived from these species would be valuable.

The emergence of antibiotic-resistant bacteria poses a significant threat, highlighting the need to discover and develop new antibacterial agents that are more effective against mutant strains and selectively target bacterial cells while sparing the host.[8]

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Antioxidants are also of great interest due to their ability to combat oxidative stress and cell damage, which contribute to numerous diseases such as cancer and coronary heart disease.[9] However, finding less toxic anticancer agents that selectively target cancer cells without affecting healthy cells has proven challenging.[10] The present study aims to explore the potential of *Phyllanthus niruri* as a novel source for identifying fungal endophytes in organic fractions, such as n-hexane, dichloromethane, and methanol. These fractions were evaluated for *in vitro* antibacterial, antioxidant, and anticancer activities, emphasizing their cytotoxic effects against 4T1 breast cancer cells. Additionally, the fungal endophyte was characterized using microscopic and molecular genetic analyses to confirm its genus.

Materials and Methods

Phyllanthus niruri L. leaves were acquired from the Materia Medica in Malang, Indonesia, the samples were collected in January 2018 with specimen code MH1223. Analytical grade chemicals such as dextrose, muller hinton, potato dextrose broth (PDB), potato dextrose agar (PDA), nutrient agar (NA), and nutrient broth were purchased from Merck. The study employed microbial strains, including *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Salmonella typhi* (*S. typhi*) from the Microbiology Laboratory at Brawijaya University, Indonesia. Chemicals used in the study comprised sodium hypochlorite (NaOCl), ethyl acetate, n-hexane, methanol, dichloromethane, hydrogen peroxide, phosphate buffer pH 7.0, potassium iron (III) cyanide, iron (III) chloride, and silica 60 for column chromatography. 4T1 cells was provided by the Parasitology Laboratory of Medical Faculty at Gadjah Mada University, Indonesia. The study also utilized various instruments such as an autoclave (AC-300AE, Tiyoda), laminar airflow cabinet (FARRco), genetic analyzer (ABI PRISM 3130 Applied Biosystems), and UV-Vis spectrophotometer (Shimadzu). Tissue culture dishes were obtained from Iwaki, and Nucleon was also used in the study.

Isolation

The method used to isolate the fungal endophyte was Kumar et al.'s, which involved eliminating epiphytic microflora and parasites from the leaf sample by surface sterilization.[11] Healthy leaves were soaked in a solution of ethanol 70% and NaOCl, rinsed in distilled water, dried on blotting paper, cut into 10 mm segments, and placed on PDA plates. The

plates were then incubated at 25°C, and after two weeks, the hyphae were inoculated on PDA plates maintained at 28°C for purification. The purity of each fungal isolate was checked, and the hyphal tip method was used for transfer. The mycelial tips were transferred into PDA plates, and the purification process was repeated five times. The mycelia were then kept in a sterile test tube slant, and one of the isolates, MCH, was selected for further molecular identification.

Molecular genetic analysis
The fungal isolates were identified through a partial genetic analysis of the fungal ribosomal DNA's Internal Transcribed Spacer (ITS) region.[12] After growing the fungal mycelia in PDB for 72 hours, they were harvested for DNA extraction.[13] Fungal DNA was extracted using Nucleon PHYTOpure reagents, and the primary ITS 4 and ITS 3 primers were used for PCR amplification. The purified PCR product underwent cycle sequencing and was then trimmed and assembled using bioedit software. The assembled sequence was compared with genomic data in DDBJ/DNA Data Bank of Japan or NCBI/National Center for Biotechnology Information using BLAST to determine the taxon/species based on the highest homology with the reference.[14]

Tissue culture
Five plugs of fungal endophyte colony, each measuring 0.5 cm in diameter, were taken from PDA. A conical flask was sterilized, and 200 mL of the colony was inoculated into it. The flask was then left to incubate at room temperature for two weeks. The mycelium was collected from the filtrate using filtration, and then dried at 50°C and pulverized.[15]

Extraction
The mycelium powder (500 g) was mixed with ethyl acetate in a 1:3 ratio and agitated regularly for 48 hours. After filtering the resulting liquid, fresh ethyl acetate was mixed with the remaining residue and agitated for another 24 hours. The filtered liquid was then collected and concentrated by reducing the pressure.[16]
Fractionation
Using an isocratic technique, 200 mL of n-hexane (100%), dichloromethane (100%), and methanol were separately used to fractionate the concentrated extract (400 mg) through a chromatography column. The fractions were then collected, concentrated under reduced pressure, and dried at 50°C.[17]

Antimicrobial test
Two methods were utilized to assess the antimicrobial activity of the samples: the disc diffusion method (Kirby-Bauer Test)[18] and the micro-dilution test[19] which was aimed at determining the minimum inhibitory concentration (MIC).

Screening of active fractions
Working solutions of different concentrations (100, 50, 25, 12.5, and 6.25 µg/mL) were prepared for each fraction in the study. These solutions were then used to prepare paper discs by dropping 10 µL of each working solution onto them, resulting in different amounts of isolate ranging from 62.5 to 1000 µg/mL. The discs were allowed to dry and attached to the media. Positive and negative controls used were streptomycin at a concentration of 10 mg/mL and sterile anhydrous ethanol, respectively. The bacterial culture was incubated at 37°C for 18-24 hours, and the inhibition zone around each paper disc was measured. The active fraction was identified by selecting the widest diameter of the observed inhibition zone.[20]

Determination of Minimum Inhibition Concentration (MIC).
For this study, a 96-microwell plate was filled with 50 µL of Muller Hinton media, followed by adding 50 µL of bacterial suspension adjusted to McFarland standard 0.5.[21] The mixture was then diluted 10 times, resulting in final concentrations of 250, 125, 62.5, 31.25, 15.63, 7.81, and 1.96 µg/mL, with streptomycin 10 mg/mL being used as the positive control. The sample was incubated at 37°C for 18-24 hours, and the cell density was measured as absorbance using a microplate reader at 595 nm. The percentage inhibition was calculated by converting the absorbance values and extrapolating them against the concentration of the active fraction. The Probit analytical method, managed by Minitab software, was used to calculate the IC_{50} of the fraction. To determine the minimum inhibitory concentration (MIC), 3 µL of the solution from each well was pipetted and streaked on NA without adding microbes and a working solution. The MIC value was defined as the concentration at which the streaked media remained clear after 18-24 hours of incubation at 37°C.[22]

Determination of a total phenolic content.
For determining the total phenolic content, each fraction was dissolved in methanol at a concentration of 2 mg/mL. Following this, 500 µL of Folin-Ciocalteu reagent (50%) and 2 mL of Na_2CO_3 20% were added to the mixture. The solution was then topped with 5 mL of distilled water and kept at room temperature for 20 minutes before measuring its absorbance at 765 nm. The total phenolic content was calculated by using the linear regression equation generated from the galic acid standard curve (30, 45, 60, 75, and 90 µg/mL) based on the absorbance value.[23]

Determination of antioxidant activities.
The fractions were subjected to two methods to determine their antioxidant activities - hydrogen peroxide free radical scavenging[24] and reducing power capacity.[25]

Hydrogen peroxide free radical scavenging
To determine the scavenging capacity of the fraction towards H_2O_2 radical, a solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer with a pH of 7.4. The fraction was dissolved in distilled water at a concentration of 2 mg/mL and then added to the H_2O_2 solution. After 10 minutes, the sample's absorbance was measured at 230 nm. The fraction's ability to scavenge the H_2O_2 radical was then calculated using a specific formula:

$$\text{Scavenging capacity } (\%) = [(\text{Ai} - \text{At})/\text{Ai}] \times 100$$

Where Ai = absorbance of the control, At = absorbance of the sample.

Reducing power capacity.
To assess the reducing power capacity of the fractions, each fraction was dissolved in 1 mL of distilled water. Then, it was mixed with 2.5 mL of phosphate buffer having a pH of 6.6 and 2.5 mL of 1% w/v potassium iron (III) cyanide. The mixture underwent centrifugation at 3000 rpm for 10 minutes, following which, the upper phase (2.5 mL) was collected and mixed with another 2.5 mL of distilled water. Further, 0.5 mL of 0.1% w/v $FeCl_3$ was added, and the absorbance was measured at 700 nm using a UV spectrophotometer.
4T1 cell assays

Cytotoxicity assay
The cytotoxicity of the fraction against 4T1 breast cancer cells was evaluated using the MTT method (26). The 4T1 and Vero cells were cultured using RPMI and M199 media, respectively, each supplemented with FBS (10%), penicillin-streptomycin (1%), and fungizone (0.5%). Working solutions of the fraction were prepared at various concentrations ranging from 7.81 to 500 µg/mL, and cisplatin (15 µM) was used as the positive control.[27] The percentage of cell viability was calculated by measuring the absorbance at 595 nm using a plate reader. The IC_{50} was determined by plotting the % viability against the concentration of the fraction. The selectivity index (SI) was defined as the ratio between the IC_{50} of the Vero cell and the IC_{50} of the 4T1 cell.[28]

Statistical analysis
The antimicrobial, antioxidant, and cytotoxic activities were evaluated in five replicates, and the results were expressed as the mean ± standard deviation (SD). To test the null hypothesis, one-way analysis of variance (ANOVA) was performed, and Tukey's test was used to compare the means of the samples. The data were considered significantly different from the positive control when the significance level was $p<0.05$.

Results and Discussions

Fungal endophyte culture

A *Cephalosporium* sp. was identified as a white fungal colony obtained from the leaves of *Phyllanthus niruri* (Figure 1a). The fungal endophyte's physical characteristics were observed, including solid helix mycelium, uniform sclerotia, and dark hyphae pigment (Figure 1b). These features were consistent with the description of the genus *Cephalosporium* sp. in the literature.[29] The Mycology Laboratory confirmed them at the Agriculture Faculty of Gadjah Mada University in Indonesia.

To identify the fungal sample at the molecular level, researchers required a universal DNA sequence barcode of a large size. The ITS gene was a useful tool in determining the systematic molecular taxonomy of the sample, including at the genus and species levels. After analyzing the rDNA gene sequences of ITS through BLAST, it was found that the sample showed 99% homology with a unique identifier for *Cephalosporium* sp. E7923 from the repository, confirming its genus as *Cephalosporium* sp. The sequence of the ITS rDNA isolate and the BLAST results for the most similar taxon are presented in Table 1.

Screening of active fraction

Identifying the fraction with the potential for further investigation required an initial evaluation of the antibacterial properties of the fractions. Table 2 presented the inhibition zone of three fractions (n-hexane, dichloromethane, and methanol) against *S. aureus*, *E. coli*, and S. *typhi*. The results showed that the dichloromethane fraction was more effective in inhibiting bacterial growth than the other two fractions. At the lowest concentration (62.5 µg/mL), the dichloromethane fraction inhibited the growth of both *S. aureus* and *S. typhi*, while the n-hexane and methanol fractions showed no inhibition. The dichloromethane fraction inhibited all bacteria equally at 125 µg/mL, and inhibition increased with increasing fraction concentration. The n-hexane and methanol fractions showed greater susceptibility to *E. coli* and *S. typhi*, respectively, at 1000 µg/mL. Although these fractions only strongly inhibited bacteria at the highest concentration, they were included in further IC_{50} calculations due to their potential antimicrobial activity against specific bacteria.

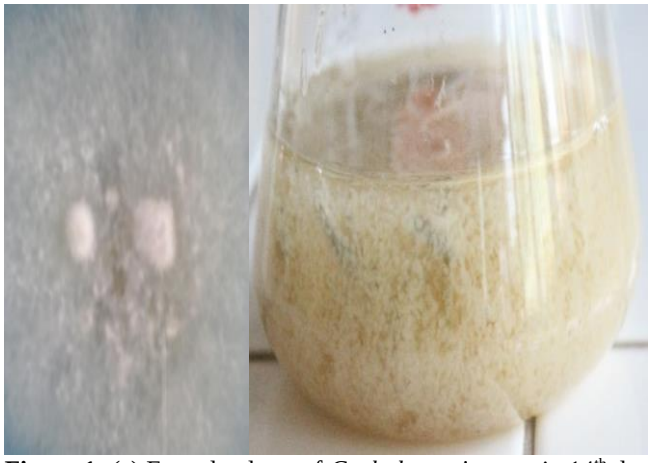

Figure 1: (a) Fungal culture of *Cephalosporium* sp. in 14th day of incubation and (b) the morphology of *Cephalosporium* sp. with 7 days in age.

Table 1: The sequence of ITS rDNA isolate and the BLAST result of the most similar coded for *Cephalosporium sp*., a spesies used as the reference. AJ8273 is the code to acces in DDBJ or NCBI

ITS_4_Reverse
CTTTAAATAGGTACTTTAGAGCCGACCTCAACTGATTCAGTACGGGACTAACGTGAACTTGTAGGACTTCGTGTGAGTTCCTTGCAA
CAGGTTAAAGTATACCAAGTTTCAAGTTTGGTACTGAACTCTTCTTATAGAAATGAGGGTCTAGGTGATAGCTGTGTTGAAAGTAC
ATCCCTGAGGATTCCTTTTGAGGTTGTGGAAGAGAAGTGTTCACTCAGTTCTAGTCCTTTAACTGGCTTAATGTACGGTATTTGAAT
AGCTCAGGGGCACTAGTTGAACGTCGTTTGAGCCTGACTAGATGTCAAGTTATGCTAGGTTACGAGTTCGGAGTCCACTCTTGCAT
CACTGTTCCTTCTGACCTTGAGCGTGAGTCTTGAAGGTTACTAGCGCGCTTTCCCGCTCGCGTTAGAACGGGGCGCTGTTGCTTGAC
TCAGAGGCTGCAATCTAGGACACTCTGCACAGGCACTAGAATAAGGATTATAGCTATAGATTCTCTAGGGCCCGTTAGCGC
ITS_5_Forward
GTAAGCTGATCTTGTAAAAAGTCAGTGACAAGCCTTCTCTTGCGTTTTACCACAAACACTTCAGTTATAGAATGTACACTGTGTATA
ACACAATATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCACTCCTTGGTATTCCGAGGAGTATGCCTGTTTGAGTCTCATGGTAT
TCTCAACCCCTGAATTTTTCTAGTAAAGATTTGGTGGGCTTGGACTTGGAGGCTGTGTCGGCTCTCGCCAGTCGACTCCTCTGAAAT
GCATTAGTGTGAACCTTACGGATCGCCTTCAGTGTGATAATT
Contig-Sample
TGACTATGCTGTACTATCTTATAGGACCTAGTGAACTAGTGCTAACGAGCCTGTACACGTGTTAGATCCACGGCTATGAGTGCTCCT
GAAGACATCTAGCAGCTTGTCAACGATCTTGCTGTTTCGAACTAGTACCTAACGGGTTAGTGGTACACTCTGTTTGCTTAACTCAGA
ACGCTACGAAAGTCACTGTTTTTGAAGTACAGCAATCAGTTCCAGGTTTAAACATAGCCGGCTTGATACCCATGACTAGTGCCTCT
GATGGGTCCCTAAAGACCTAACGAGCCTATCAAGTTCGGCCTGGATGTCTTTAAGTACCTCTAAAGTTTGCTAGTTCCCTGGAGGTT
ATCCACTGACCTAGTGTTGGTCAGAAACAGAACCTGTTATCTCTCCAGGCTAGGTGTCACGGTGAGAACGAGGACCTCTGTTACCT
CCGGTTTCATTATGT
Cephalosporium sp.
(Accession no: AJ8273) [Homology: 97%; Max score: 1089; Total score: 1089; Query coverage: 100%; E-value: 0.0; Max identities: 566/567 (99%); Gaps: 1/567 (0%)]

Table 2: The results of the disc diffusion assay of the fractions against the bacteria.

Fraction	Loading (µg)	Growth inhibition zone (mm)				
		S. aureus	*E. coli*	*S. thypi*	(+) control	(-) control
n-hexane	62.5	1	ND	ND		
	125	2	2	1		
	250	2	3	1	20	ND
	500	3	4	2		
	1000	5	7	3		
Dichloromethane	62.5	1	ND	2		
	125	2	3	3		
	250	3	3	7	19	ND
	500	8	6	8		
	1000	17	8	11		
Methanol	62.5	ND	ND	ND		
	125	ND	ND	2	21	ND
	250	ND	1	4		
	500	1	2	7		
	1000	2	4	11		

ND = not detected

Table 3: The IC_{50} and MIC value of three fractions against S. *aureus*, *E.coli* and *S.typhi*.

Bacteria	Samples							
	n-Hexane		Dichloromethane		Methanol		Streptomycin	
	IC_{50} (µg/mL)	MIC (µg/mL)	IC_{50} (µg/mL)	MIC (µg/mL)	IC_{50} (µg/mL)	MIC (µg/mL)	IC_{50} (µg/mL)	MIC (µg/mL)
E. coli	128.83 ± 2.3*	500*	17.23 ± 0.93*	112.93 ± 1.32*	112.93 ± 2.36*	250*	0.93 ± 0.01	0.82 ± 0.03
S. aureus	272.93 ± 31.32*	500*	78.93 ± 1.23*	119.12 ± 3.21*	119.12 ± 2.12*	250*	0.21 ± 0.09	1.23 ± 0.02
S. typhi	231.02 ± 1.1*	500*	12.39 ± 0.23*	89.25 ± 4.21*	89.25 ± 1.32*	250*	0.72 ± 0.05	1.93 ± 0.03

Values area means ± SD, n = 5 replicates, $p<0.05$ indicates statistically significant differences in comparison to streptomycin control.

Minimum Inhibition Concentration (MIC)

The study selected the most potent fraction based on its ability to inhibit bacterial growth at the lowest concentration. If the inhibition rate was at least 50%, only the IC_{50} value was determined. Table 3 shows each fraction's MIC and IC_{50} values against three types of bacteria. The dichloromethane fraction exhibited the highest efficacy of all fractions, as it inhibited all bacteria at a lower concentration than the other two fractions. The dichloromethane fraction had an IC_{50} value of less than 100 µg/mL against all bacteria, indicating its broad-spectrum activity. Among the three bacteria, *S. typhi* was the most susceptible to the dichloromethane fraction, with the lowest IC_{50} value (12.39 µg/mL). The n-hexane and methanol fractions were less sensitive than the dichloromethane fraction, with IC_{50} values of over 100 µg/mL. However, the methanol fraction selectively inhibited *S. typhi* more than other bacteria in the initial antibacterial assay due to its IC_{50} value of 89.25 µg/mL, indicating its potential as a narrow-spectrum antibacterial. The least effective fraction was n-hexane, with IC_{50} values of more than 100 µg/mL against all bacteria.

The total phenolic content

The total phenolic content of compounds is linked to their ability to reduce redox reactions. Table 4 illustrates that the three fractions had varying total phenolic content. Phenolic compounds were more soluble in polar solvents like methanol due to their polar nature, as opposed to semipolar (dichloromethane) and nonpolar (n-hexane) solvents. However, the dichloromethane fraction had a relatively high total phenolic content, contributing to its antibacterial activity. Phenol can denature proteins and eliminate bacteria, making it effective against them.[30]

Antioxidant activity

Hydrogen peroxide can produce hydroxyl radicals that can damage cells by altering the lipid peroxidation process. All three fractions in this study demonstrated free radical scavenging activity ranging from 34-85% (Figure 2a). The methanol fraction exhibited the highest scavenging activity (86%), comparable to that of vitamin C (98%), the positive control. This result was consistent with the reducing power capacity assay (Figure 2b), which indicated that the methanol fraction could donate electrons to stabilize radicals up to 80%, almost equivalent to vitamin C (97%). These two antioxidant activities correlated with the total phenolic content, as polar solvents like methanol effectively extracted phenolic compounds. The antioxidant activity of the methanol fraction may be linked to the inhibition of lipid peroxidation.

Cytotoxicity assay

The 4T1 cell line is a commonly used breast cancer cell line in breast cancer research.[31] The fractions tested in this study exhibited potential anticancer activity, with the ability to inhibit the growth of 4T1 cells by up to 50% (Table 5). The dichloromethane fraction had the lowest IC_{50} value and the highest selectivity index for cancer cells over normal cells.[32] These findings were consistent with the results of the antibacterial and cytotoxicity assays, indicating that this fraction may have potential as a broad-spectrum chemotherapeutic agent against bacteria and cancer cells.[33] Figures 3 (a and b) illustrate the concentration of the dichloromethane fraction plotted against the percentage inhibition of 4T1 and Vero cells, respectively. Exposure to the dichloromethane fraction caused 4T1 cells to shrink, die, and reduce in number, as seen in Figure 4a-c. In contrast, the cytotoxic effects of

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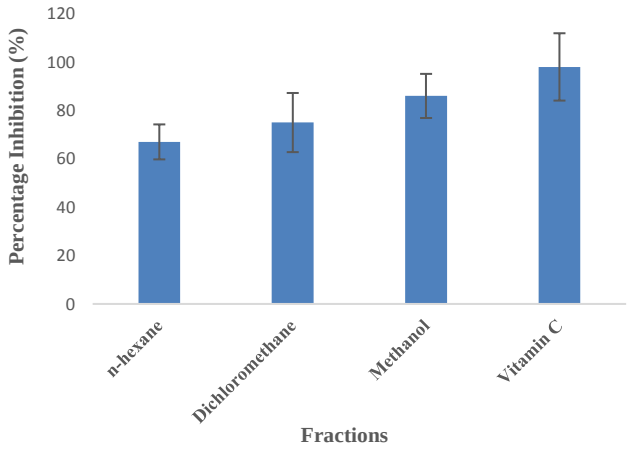
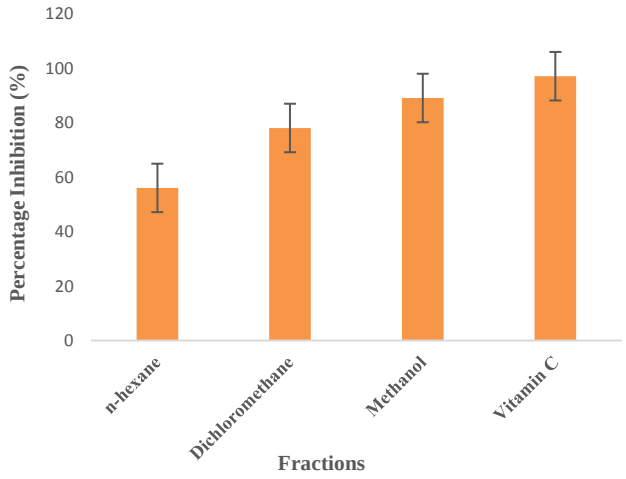
the dichloromethane fraction on Vero cells were milder than those on 4T1 cells, although exposure caused Vero cells to shrink, circle, and detach from the well (Figure 4d-f).

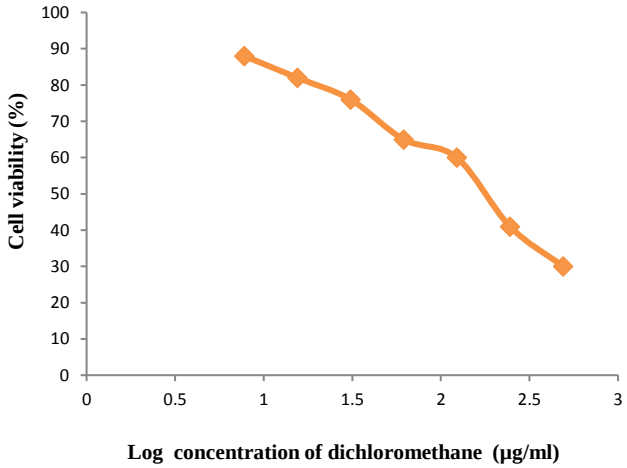


(a)

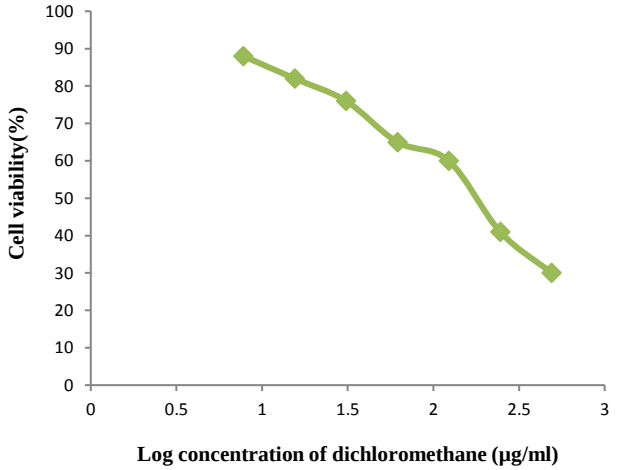


(b)

Figure 2: The antioxidant activity of three fractions using methods: (a) H_2O_2 free radical scavenging and (b) reducing power capacity.



(a)



(b)

Figure 3: The drug-dose response curve of dichloromethane fraction against the percentage viability of (a) 4T1 and (b) Vero cell with $p < 0.05$.

Table 3: The IC_{50} and MIC value of three fractions against S. *aureus*, *E.coli* and *S.typhi*.

Bacteria	Samples							
	n-Hexane		Dichloromethane		Methanol		Streptomycin	
	IC_{50} (µg/mL)	MIC (µg/mL)	IC_{50} (µg/mL)	MIC (µg/mL)	IC_{50} (µg/mL)	MIC (µg/mL)	IC_{50} (µg/mL)	MIC (µg/mL)
E. coli	128.83 ± 2.3*	500*	17.23 ± 0.93*	112.93 ± 1.32*	112.93 ± 2.36*	250*	0.93 ± 0.01	0.82 ± 0.03
S. aureus	272.93 ± 31.32*	500*	78.93 ± 1.23*	119.12 ± 3.21*	119.12 ± 2.12*	250*	0.21 ± 0.09	1.23 ± 0.02
S. typhi	231.02 ± 1.1*	500*	12.39 ± 0.23*	89.25 ± 4.21*	89.25 ± 1.32*	250*	0.72 ± 0.05	1.93 ± 0.03

Table 4: The total phenolic content of *n*-hexane, dichloromethane, and methanol fractions

Fraction	Total Phenolic Content (mg GAE/g Fracton)
n-hexane	87.23 ± 0.83
Dichloromethane	17.21 ± 0.23
Methanol	19.12 ± 0.73

Values area means ± SD, n = 5 replicates

Conclusion

Cephalosporium sp. endophyte from *Phyllantus niruri* L. was evaluated for antimicrobial, antioxidant, and cytotoxic activities in various organic fractions. The most effective fraction against bacteria and breast cancer cells was dichloromethane, while methanol showed the highest antioxidant activity.

Conflict of Interest

The authors declare no conflict of interest.

Table 5: The results of cytotoxicity assay against 4T1 cell line

Fraction	IC_{50} 4T1 (µg/ml)	IC_{50} Vero (µg/ml)	Selectivity index (SI)
n-hexane	92.23 ± 0.23	372.83 ± 2.39	4.04
Dichloromethane	18.12 ± 0.92	526.42 ± 0.82	29.05
Methanol	52.12 ± 1.93	72.23 ± 2.32	1.38

Values area means ± SD, n = 5 replicates

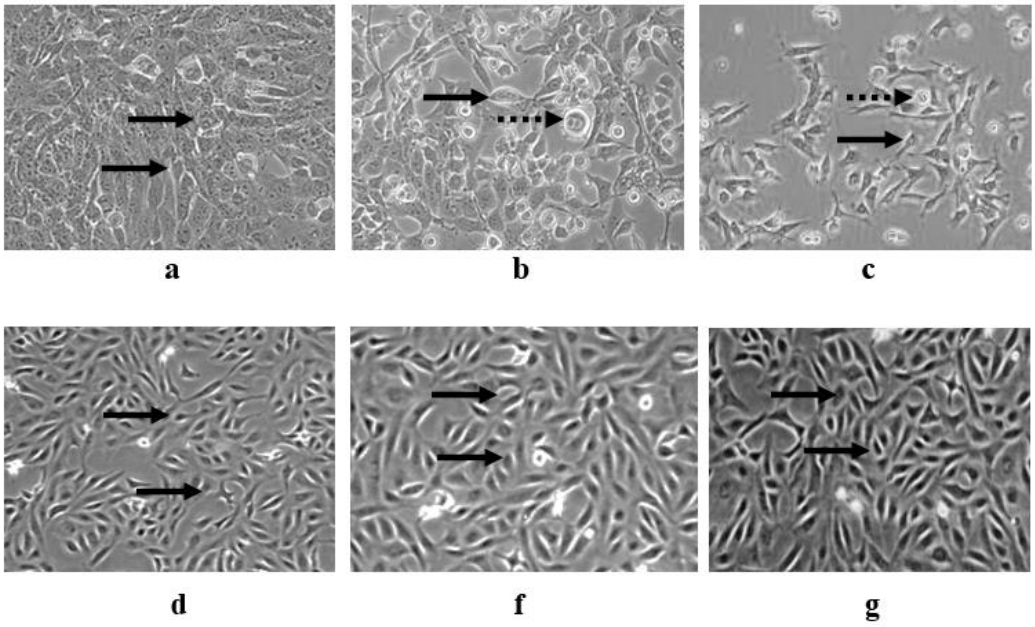


Figure 4: The effect of dichloromethane fraction towards 4T1 (a-c) and Vero cells (d-f). The viewing was performed under inverted microscope with 100x of magnitudes. (a) negative control (b) dichloromethane fraction 5 µg/mL, (c) 10 µg/mL, (d) negative control, (e) dichloromethane fraction 250 µg/mL and (f) dichloromethane fraction 500 µg/mL. The arrow indicated the living cell whereas the dashed arrow indicated the morphological alteration.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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