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Antibacterial and Anti-biofilm-Forming Activity of Secondary Metabolites from *Sansevieria trifasciata* Leaves Against *Pseudomonas aeruginosa*

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ABSTRACT

Sansevieria trifasciata is an herbaceous plant frequently used in traditional medicine with antibacterial potency against *Pseudomonas aeruginosa*. *P. aeruginosa* is a known bacterial pathogen that often becomes resistant to traditional antibiotics due to its ability to form biofilms. The objective of this research was to determine whether the ethanolic extract of *S. trifasciata* has any antibacterial or anti-biofilm-forming activity against *P. aeruginosa*. Bacterial cell damage with a potential fraction of *S. trifasciata* ethanolic extract treatment was observed by scanning electron microscopy (SEM). An anti-biofilm assay was performed in a 96-well microtiter plate with crystal violet stain. The minimum inhibitory concentration (MIC) results revealed that at a concentration of 4 mg/mL, the potential fraction of *S. trifasciata* ethanolic extract could inhibit bacterial growth. At this concentration, the potential fraction of *S. trifasciata* ethanolic extract could inhibit biofilm formation by 60%. The identification of compounds using gas chromatography–Mass Spectrometry (GC-MS) revealed that Neophytadiene was the main active component in the extract. These compounds have the potential to be used as therapeutic agents in the prevention of bacterial biofilms and virulence-related infectious illnesses.

Key words: *S. trifasciata*, *P. aeruginosa*, antibacterial, anti-biofilm

INTRODUCTION

Sansevieria trisfasciata is a plant that has been known and cultivated as an ornamental plant since the 19th century. In addition to its use as an ornamental plant, the leaves are also employed in traditional medicine to treat diarrhea, coughs, respiratory tract inflammation, and hair growth. In Africa, latex from these plants can be used as a snake and insect repellent (Lu & Morden, 2014; Umoh *et al*., 2020). An antibacterial agent is also sourced from *S. trifasciata* Prain. In tropical nations, *S. trifasciata* is used to treat inflammatory diseases and is offered on the market as a crude treatment for snakebite, as well as ear ache, swellings, boils, and fever (Anbu *et al*., 2009). The pharmacological activities of *S. trifasciata* have been investigated, including its antioxidant, anticancer, antidiabetic anaphylactic, and antibacterial properties. Several studies have indicated that *S. trifasciata* leaves have antibacterial activity against various bacteria, including *Pseudomonas. aeruginosa* (Andhare *et al*., 2012; Berame *et al*., 2017; Dewatisari *et al*., 2021; Kingsley *et al*., 2013).

P. aeruginosa is a common organism and the subject of extensive research due to its importance in disease. Pseudomonas aeruginosa is an important opportunistic pathogen distinguished by its ability to form biofilms and high levels of multiple antibiotic resistance. Furthermore, *P. aeruginosa* infections can be difficult to eradicate due to the high prevalence of antibiotic resistance and biofilm formation among them (Dzen, 2003). A biofilm is a group of microbial cells encased in a matrix of extracellular polymeric material. Quorum sensing enables biofilm members to converse, communicate, and exchange virulence factors with one another. Bacterial biofilm development is a key element in pathogenicity and survival. Biofilms of *P. aeruginosa* may influence the clinical outcomes

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of individuals with chronic wounds. *P. aeruginosa* is the most threatening of the infectious microorganisms that form biofilms in the human host. Antimicrobials are ineffective at controlling biofilms and do not provide enough information on biofilm dispersion. The purpose of recent research is to develop novel chemicals that can prevent biofilm formation (Dolatabadi *et al.*, 2018; Purwestri *et al.*, 2016; Retnaningrum *et al.*, 2020; Retnaningrum & Wilopo, 2018; Sathiya Deepika *et al.*, 2018).

Medicinal herbs have been utilized as traditional remedies for human infections for thousands of years. Their extracts play a key role in the treatment of biofilm-related disorders, and they are generally less toxic and cause fewer adverse effects than synthetic drugs. In addition to their potential antibacterial effects, promising candidate plants capable of interfering with the bacterial communication system, and ultimately the pathogenesis of bacteria, without affecting their viability, will have essential therapeutic value in reducing *P. aeruginosa* infection. For ages, several plant-based medications have been used to treat bacterial diseases and reduce inflammation. They are thought to have low toxicity, making them suitable for therapeutic use (Călina *et al.*, 2017; Dolatabadi *et al.*, 2018; Ginting *et al.*, 2021; Kim *et al.*, 2015).

This resistance mechanism necessitates the development of new antibiotics for infection treatment. One solution to this problem is the use of natural remedies from plants. Although several articles demonstrate the growth-inhibiting effect of *P. aeruginosa* from *S. trifasciata* extracts, studies relating to *S. trifasciata*-mediated anti-biofilm activity have not been conducted on *P. aeruginosa*. The extraction should be simplified into a fractionation process that will produce bioactive compounds from more specific fractions of *S. trifasciata* ethanolic extract.

Phytochemical substances found in the leaves of *S. trifasciata* extracted using ethanol solvent include alkaloids, flavonoids, saponins, glycosides, terpenoids, tannins, proteins, and carbohydrates. Several investigations have shown that the ethanolic extract of *S. trifasciata* leaves has analgesic and antipyretic properties, as well as antibacterial and antioxidant properties. Quinolones, 3,4-dimethoxybenzoic acid, palmitaldehyde, 1,2-benzene-dicarboxylic acid, linoleic acid, quinolone, and delta-undecalactone are some of the bioactive chemicals found in leaf extracts of *S. trifasciata* that are responsible for its antibacterial activity (Abdullah *et al.*, 2018; Dewatisari *et al.*, 2021; Yumna *et al.*, 2018). Researchers are interested in conducting antibacterial tests against a probable fraction of *S. trifasciata* ethanolic extract due to a variety of chemical components it may contain. This is the first study to report the identification of bioactive compounds from the potential fraction of *S. trifasciata* leaves.

The purposes of this study were as follows: (i) to evaluate the antibacterial and anti-biofilm capabilities of the potential fraction of *S. trifasciata* ethanolic extract against *P. aeruginosa*; (ii) to observe cell damage with SEM following treatment with a potential fraction of *S. trifasciata* ethanolic extract; and (iii) to identify the active compounds associated with the antibacterial activity of *S. trifasciata* ethanolic extract.

MATERIALS AND METHODS

The materials utilized in this research were the potential fraction of *S. trifasciata* leaves and *P. aeruginosa* FBGMU 01 bacteria. The dried powder of *S. trifasciata* leaves was extracted using a maceration method with chloroform and then with ethanol. Plant materials and solvent were mixed in a 1:3 (w/v) ratio. The potential fraction of *S. trifasciata* leaves resulted from fractionation analysis using vacuum liquid chromatography from the ethanolic extract. The same fraction pattern was combined into one fraction using thin-layer chromatography (TLC). The fraction with the highest antibacterial activity was selected as the potential fraction (Dewatisari *et al*., 2021). The research was conducted at the Microbiology, Biochemistry, and Joint Research Facility Laboratory, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Fractionation Using Preparative Thin-Layer Chromatography (TLC) of Potential Fractions of *S. trifasciata*

The potential ethanolic extract fraction was re-fractionated using TLC preparative analysis. The fraction was smeared on silica gel 60 GF254 (Merck) and developed in 3:2 ethyl acetate: methanol (v/v). The layers formed were scraped from the plate, extracted into ethanol, and filtered. The fractions were then tested for antibacterial activity using a disk diffusion assay.

Antibacterial activity determination

P. aeruginosa FBGMU 01 was inoculated into Nutrient Agar media using the pour plate method

with a cell concentration of 1.5 × 10^8 CFU/mL (0.5 McFarland). The fraction of *S. trifasciata* ethanolic extract was present in paper disks at a concentration of 32 mg/mL. The diameter of the inhibitory zone was measured after 24 h of incubation to determine antibacterial activity. The result of the largest diameter produced was the potential fraction. The potential fraction of *S. trifasciata* ethanolic extract was then analyzed for minimum inhibitory concentration (MIC), anti-biofilm, and SEM and its compounds identified by GC-MS.

Determination of Minimum Inhibitory Concentration (MIC)

The potential fraction of *S. trifasciata* ethanolic extract concentrations used were 4–256 mg/mL. In a test tube, 200 μL of bacterial culture with a cell concentration of 1.5 × 10^8 CFU/mL was inoculated with 8.8 mL of nutrient broth (NB). *P. aeruginosa* growth was observed after 24 h of incubation at 37°C, and its optical density (OD) was measured using an ELISA Reader at a wavelength of 595 nm (Arung *et al.*, 2017; Balouiri *et al*., 2016).

Biofilm Inhibition assay

A 96-well microtiter plate with crystal violet staining was used for the biofilm inhibition test. A 100-μL volume bacterial suspension with a cell density of 1.5 × 10^8 CFU/mL (0.5 McFarland) containing 1% glucose and 1% ammonium sulfate was dispensed into each well of a 96-well microplate. Then, a potential fraction of *S. trifasciata* ethanolic extract was added with an MIC of 100 μL. The negative control used was 200 μL of NB without bacterial suspension and ciprofloxacin. In comparison, the positive control was a bacterial suspension without addition of 200 μL potential fraction of *S. trifasciata* ethanolic extract and ciprofloxacin. The treatment was performed in three replications and then incubated for 48 h.

The 96-well microplates were rinsed three times with running water after the incubation period, then 200 μL of 1% crystal violet was added to each well and incubated at room temperature for 15 minutes. The 96-well microplates were washed three additional times in running water. Each well was then filled with 200 μL of 96% ethanol solution and incubated for another 15 minutes at room temperature. Furthermore, the OD was measured using an ELISA reader at a wavelength of 630 nm. Each sample was tested for biofilm production in triplicate. The purpose of this study was to determine whether a potential fraction of *S. trifasciata* ethanolic extract could inhibit the growth of *P. aeruginosa* biofilm. A three-level scale was used to assess biofilm formation ability: very positive: OD > 1,500; positive: OD 0.500–1,500; and negative: OD = 0.500. All experiments were conducted in triplicate. The biofilm formation was determined using an OD cut-off (ODC) greater than OD630 = 0.03. The following criteria were used to calculate the results:

$$\text{biofilm formation inhibition (\%)} = 100 \times \frac{OD_c - OD_s}{OD_c}$$

OD*c*: OD control; OD*s*: OD sample;
where control represents the OD of untreated test bacteria and OD sample represents the OD of test bacteria treated with possible fraction of *S. trifasciata* ethanolic extract (Dolatabadi *et al.*, 2018).

Analysis by Scanning Electron Microscopy (SEM)

SEM was performed in accordance with the method described by Ginting *et al*. (2021) and Özogul *et al*. (2020), with the purpose of examining the different effects of normal *P. aeruginosa* and *P. aeruginosa* treated with a potential fraction of *S. trifasciata* ethanolic extract on morphological changes in bacterial cell walls. Normal *P. aeruginosa* and *P. aeruginosa* treated with a potential fraction of *S. trifasciata* ethanolic extract were added to a broth culture at the MIC of each tested bacteria and incubated at 37°C for 24 hours. Each tube was centrifuged for 10 minutes at 4000 *g* at 4°C after incubation. The samples were rinsed twice in distilled water and then resuspended in 1 mL water. Subsequently, 10 μL of the suspension was applied to the 1 x 1 centimeter slide. This sample was then dehydrated with 10% ethanol in a vertical laminar flow biological cabinet (Telstar Class II Cabinet) at 25°C. The prepared sample was placed in a desiccator until it was coated with gold in a sputter ion coating (Quorum Technologies Ltd.,) for 10 minutes, after which it was examined using a SEM (Quanta 650 Field Emission SEM, FEI).

Identification of Bioactive Compounds Using GC-MS

The potential fraction of *S. trifasciata* ethanolic extract was dissolved in 1 mL of ethanol. The solution was then filtered, and 0.5 μL of it was injected into a gas chromatograph (Shimadzu GC-MS-QP2010S), for which a glass column 30 m long, 0.25 mm in diameter, and 0.25 m thick was used. The stationary phase used was CP-Sil 5 CB, with a

programmed oven temperature of 70–260°C, a temperature rise rate of 10°C/min, a helium carrier gas at a pressure of 12 kPa, a total rate of 50 mL/min, and a split ratio of 1:50.

Data analysis

All measurements were conducted three times. The data was evaluated both qualitatively and quantitatively. The data were processed with SPSS 26 and evaluated with one-way analysis of variance and the Tukey test at a 95% confidence level and a significance value of $p < 0.05$.

RESULTS AND DISCUSSION

Antibacterial activity

The potential fraction was separated into four fractions using preparative TLC. (Figure 1). Disc diffusion was used to investigate the bactericidal activity of the four fractions. The inhibition zones were fraction 4 (9.50 mm), fraction 2 (4.50 mm), fraction 3 (4.40 mm), and fraction 1 (0.00 mm) (Table 1). Fraction 4 of *S. trifasciata* showed the highest antibacterial activity (Figure 2).

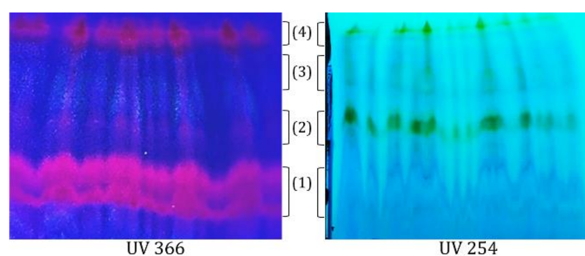


Figure 1. Results of Preparative TLC with four layers of fractions observed under UV 366 and UV 254: (1). fraction 1, (2). fraction 2, (3). fraction 3, (4). fraction 4.

Table I. Antibacterial activity of four TLC preparative fractions.

Fractions	Inhibition Zones (mm)
1	5.50±0.00^{a}
2	4.50±0.50^{b}
3	4.40±0.36^{b}
4	9.50±0.50^{c}

Note: * The antibacterial activity values are shown by the mean ± standard error from three replications. Different superscript alphabetic letters differed considerably, according to the Tukey test ($p < 0.05$).

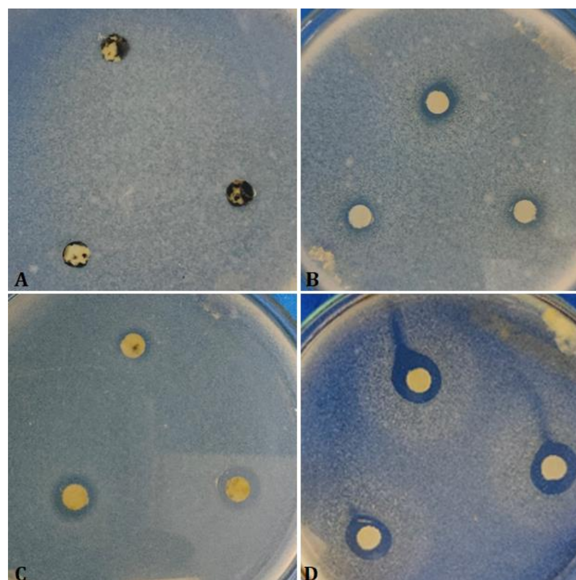


Figure 2. The antibacterial activity of fractions of *S. trifasciata* leaf ethanolic extract against P. aeruginosa: (1). fraction 1, (2). fraction 2, (3). fraction 3, (4). fraction 4

Table II. Effect of fraction 4 concentration on bacterial growth: determination of MIC

Fraction Concentration	Optical Density ± SD
NB & bacteria (+)	0.902±0.16^{c}
4 mg/mL	0.363±0.06^{b}
8 mg/mL	0.378±0.07^{b}
16 mg/mL	0.246±0.05^{ab}
32 mg/mL	0.058±0.02^{a}
64 mg/mL	0.061±0.02^{a}
128 mg/mL	0.057±0.02^{a}
256 mg/mL	0.066±0.02^{a}
NB (-)	0.047±0.00^{a}

Note: * The optical density values are shown as the mean ± standard error from three replicates. Different superscript alphabetic letters differed significantly according to the Tukey test ($p < 0.05$).

Minimum Inhibitory Concentration (MIC)

Fraction 4 had the strongest antibacterial activity, according to the results of preparative TLC analysis (Figure 2). This potential fraction was then tested by MIC to determine the lowest concentration that inhibited bacterial growth by 50% from a concentration of 256 mg/mL to 4 mg/mL. The qualitative test was carried out by

observing the turbidity of the macrodilution method. Quantitative tests were conducted by measuring the OD at each concentration. The lowest concentration that inhibited bacterial growth, as seen from the turbidity observation, was 4 mg/mL (fraction 4). At this concentration, the bacterial population was reduced by 50% in an OD measurement using an ELISA Reader. Thus, the MIC potential fraction of *S. trifasciata* ethanolic extract was 4 mg/mL (Table II).

Biofilm inhibition

The test aimed to determine the ability of the potential fraction of ethanolic extract from *S. trifasciata* leaves to inhibit biofilm by means of crystal violet staining using a 96-well microtiter plate. The light purple wells represent biofilm-negative samples (OD ≤ 0.500), and the darker wells represent moderate and vigorous biofilm producers (0.500 ≤ OD ≤ 1.500) (Figure 3 and Table III). Bacteria with a potential fraction of *S. trifasciata* ethanolic extract had an average OD of 0.40, which means that the resulting biofilm was relatively weak. These results were not significantly different from those obtained with ciprofloxacin and media without bacteria.

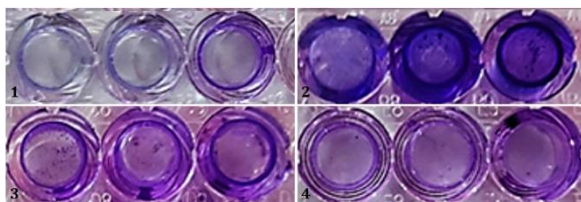


Figure 3. Microtiter plate assay indicating biofilm production with crystal violet: (1). NB, (2). NB & bacteria, (3). potential fraction of S. trifasciata ethanolic extract, (4). Ciprofloxacin.

Table III. Biofilm inhibition test

Materials	Optical Density ±SD
NB	0.04 ± 0.00^{a}
NB & bacteria	1.19 ± 0.21^{b}
Potential fraction of *S. trifasciata* ethanolic extract	0.40 ± 0.27^{a}
Ciprofloxacin	0.10 ± 0.04^{a}

Note: * The optical density values are shown by the mean ± standard error from three replications. Different superscript alphabetic letters differed considerably, according to the Tukey test ($p < 0.05$).

A bacterial biofilm is a bacterial population that adheres to a surface and is immersed in a matrix of bacterial-derived organic polymers. This was the first study to investigate the anti-biofilm activity of the potential fraction of *S. trifasciata* ethanolic extract. The inhibition of biofilm formation showed that the additional potential fraction of *S. trifasciata* ethanolic extract could affect *P. aeruginosa* biofilms. The comparison of biofilm formation between positive and negative controls showed that biofilm inhibition by the potential fraction of *S. trifasciata* ethanolic extract was 60% lower than that of the control (without potential fraction of *S. trifasciata* ethanolic extract).

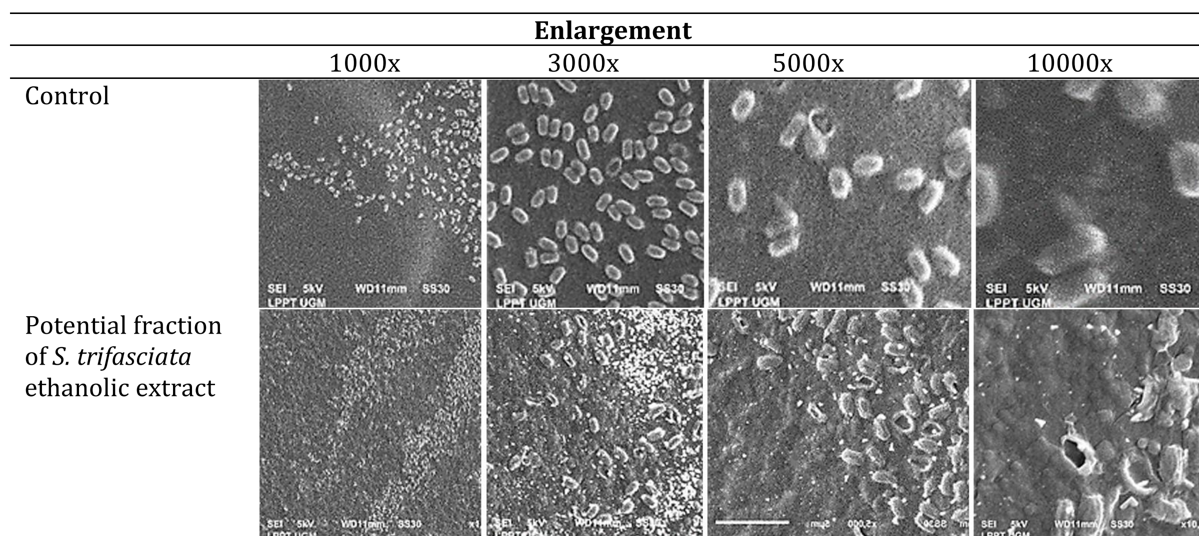
Several anti-biofilm studies using plant extracts against *P. aeruginosa* have been conducted. With an average MIC value of 16 mg/mL, *Juglans regia* L. leaves extract effectively inhibited biofilm formation (Dolatabadi *et al*., 2018). The chemical properties of the walnut tree is shown to be responsible for its antimicrobial effects against pathogens. The leaves of *J. regia* L. contain monoterpenes and sesquiterpenes, and the bark contains ketones such as juglone, phenolic compounds, regiolone, sterol, and flavonoids (Carvalho *et al*., 2013).

At MICs of 100 g/mL and 75 g/mL, respectively, Namasivayam *et al*. (2013) observed that *Acacia indica* and *Vitex negundo* exhibited a maximum percentage of biofilm inhibition of 65% and 51%, respectively. A study investigating the effect of pomegranate alcohol extract on *P. aeruginosa* biofilms in humans demonstrated that biofilms were removed in 50% to 95% of cases (Mashhady *et al*., 2016). *Mangifera indica* leaf extract could reduce *P. aeruginosa* PAO1 biofilm formation by over 36%–82% (Husain *et al*., 2017). The potential of plant extracts to inhibit bacterial biofilms has been previously reported, suggesting that using bioactive plant extracts to reduce microbial colonization on epithelial surfaces or mucosa, which leads to infection, could be a viable approach (Quave *et al*., 2008; Stefanovic, 2018; Teanpaisan *et al*., 2017)

SEM analysis results

Observation with SEM aimed at studying the pattern of changes in morphology and structure of bacterial cells under the influence of the potential fraction of *S. trifasciata* ethanolic extract. Changes that can be observed are patterns of damage to morphology and structure of bacteria, namely general appearance, cell size, damage to cytoplasmic walls and membranes.

Table IV. SEM Image of *P. aeruginosa* treated with the potential fraction of *S. trifasciata* ethanolic extract

	Enlargement			
	1000x	3000x	5000x	10000x
Control				
Potential fraction of *S. trifasciata* ethanolic extract				

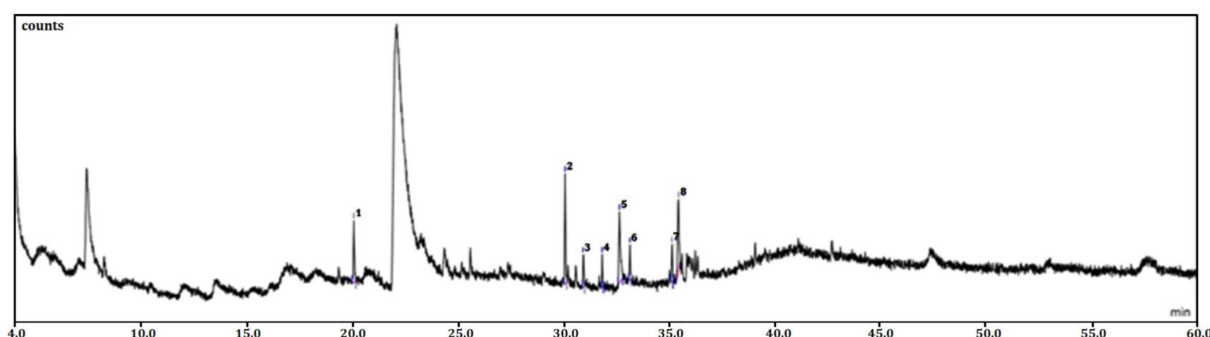


Figure 4. GC-MS chromatogram of chemical components of the potential fraction of *S. trifasciata* ethanolic extract.

SEM was used to observe morphological changes in each tested bacterial cell. Untreated bacteria had normal cell shape, whereas bacteria treated with a potential fraction of *S. trifasciata* ethanolic extract had shrinkage and cell lysis (Table IV).

Bacterial damage is caused by the interaction of antibacterial compounds with certain parts of bacterial cells (Zhanel *et al*., 1992). *P. aeruginosa* cell walls treated with potential fraction of *S. trifasciata* ethanolic extract caused the bacterial cell walls to be hollow compared with untreated cells. The formation of this whole is due to disruption of the cell membrane and changes in cell permeability which in turn causes the release of cell material out. The interaction of these antibacterial compounds can lead to several changes or damage to bacterial cells that affect the pattern of bacterial inactivation. At doses that are not lethal, the bacteria will experience injury; there will be several changes and damage to the bacterial cell structure, ultimately affecting cell metabolism function; in severe damage, it will cause death. The shape and magnitude of changes or damage to cell structure are affected by the antibacterial compound, bacteria, and concentration used. Changes and damage to cell structure by antibacterial compounds can affect cell morphology, cell structure, cell size, leakage of cell walls and membranes, wall thickness, and cytoplasmic appearance (Ash & Ash, 1997).

Table V. The chemical components of a potential fraction of *S. trifasciata* ethanolic extract were analyzed using GC-MS.

Peak	Retention time	Area	Area%	Formula	Compound name
1	20.07	138145.131	1322	$C_{13}H_{18}O_2$	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl
2	30.07	231051.083	22.12	$C_{20}H_{38}$	Neophytadiene
3	30.92	64082.899	6.13	$C_8H_{16}O_8$	3-Trifluoroacetoxypentadecane
4	31.82	54379.703	5.21	$C_{24}H_{46}O_2$	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester
5	32.63	208748.754	19.98	$C_{10}H_{17}NO_6S$	n-Hexadecanoic acid
6	33.14	61672.807	5.90	$C_{22}H_{44}O_3$	Hexadecanoic acid, ethyl ester
7	35.13	66142.692	6.33	$C_{24}H_{46}O_2$	11-Octadecenoic acid, methyl ester
8	35.44	220379.458	21.10	$C_{16}H_{30}O_2$	2-Myristynoyl pantetheine

Inhibition of bacterial activity by plant bioactive components can be caused by several factors, including (1) disturbances in the compounds that make up the cell wall, (2) increased permeability of cell membranes, which causes loss of constituent cell components, (3) inactivation of metabolic enzymes, and (4) destruction or malfunction of genetic material (Zhang *et al*., 2012). According to Kanazawa *et al*. (1995), the above process is caused by antimicrobial compounds adhering to the surface of microbial cells or diffusing into the cells.

GC-MS analysis results

The chromatogram with separate peaks based on retention time (RT) and percentage area (Figure 4). Lists the phytoconstituents found in the potential fraction of *S. trifasciata* ethanolic extract, together with their chemical structures, as determined by GC-MS peak reports of total ion chromatogram (Table V).

The GC-MS analysis revealed a total of eight different compounds from the potential fraction, as listed in Table 5 and the chromatograms in Figure 4, where Neophytadiene accounts for the main proportion up to 22.12% with a RT of 30.07 minutes. Neophytadiene showed the highest area, followed by 2-Myristynoyl pantetheine (21.10%), n-Hexadecanoic acid (19.98%), 2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl (13,22%),11-Octadecenoic acid, methyl ester(6,33%), 3-Trifluoroacetoxypentadecane (6,13%), Hexadecanoic acid, ethyl ester(5,90%), Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-,methyl ester (5.21%). Antimicrobial, anticancer, antiarthritic, anti-inflammatory, and antiviral activities have been documented for these phytoconstituents (Olajuyigbe *et al.*, 2018; Painuli *et al.*, 2015).

The antibacterial activity of neophytadiene has been reported to be broad-spectrum. Furthermore, Neophytadiene has been expressed from various plant sources with antimicrobial and anti-biofilm properties, which have previously been reported to interfere with QS-mediated virulence factor production and biofilm formation in *P. aeruginosa* (Baloyi *et al*., 2021).

Neophytadiene can inhibit the growth of or kill bacteria by interfering with membrane formation or causing cell walls to form imperfectly (Ajizah, 2004). In addition, (Cowan, 1999) stated that terpenoids exert antibacterial activity by forming strong polymer bonds with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, thereby destroying porins. Damage to the porin, which serves as the compound's entrance and exit, reduces the permeability of the bacterial cell wall. This cell wall permeability will interfere with the entry and exit of nutrients and other compounds, inhibiting or halting bacterial growth.

Neophytadiene is a terpenoid from the diterpene group, which is widely contained in essential oils. Essential oils are the essence of medicinal plants. Chemical compounds in plants can inhibit the growth of microorganisms by hampering the synthesis of cell walls and nucleic acids or preventing protein synthesis. The antibacterial activity of the volatile compound

Neophytadiene found in the ethanolic extract of *Zingiber cassumunar* leaves is well known. The antibacterial mechanism of Neophytadiene (diterpene) is not known with certainty. However, it was estimated that terpenes could damage bacterial membranes with lipophilic compounds (Stefanovic, 2018). Small amounts of terpenoids and phenolic compounds in herbal plants are known to destroy the outer membrane of gram-negative bacteria (Quinto *et al*., 2019). Neophytadiene is a potent antimicrobial, antifungal, antioxidant, and anti-inflammatory compound (Venkata raman *et al*., 2012)

This is the world's first report on GC-MS analysis of the potential fraction of *S. trifasciata* ethanolic extract. Several bioactive and industrially significant chemicals have been discovered in ethanolic extracts of *S. trifasciata* leaves.

CONCLUSION

In this study, the potential fraction of *S. trifasciata* ethanolic extract showed its ability as an antibacterial and anti-biofilm-forming agent against *P. aeruginosa*. Neophytadiene was identified as the dominant compound in the potential fraction of *S. trifasciata* ethanolic extract, which effectively reduced biofilm formation. SEM observations showed shrinkage and cell lysis in bacteria treated with the potential fraction of *S. trifasciata* ethanolic extract. Therefore, *S. trifasciata* leaves could be used as good candidate agents from plants for reducing the pathogenicity and resistance of *P. aeruginosa.*

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