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RESEARCH ARTICLE

Identification of antibacterial compounds in *Dracaena trifasciata* (Prain) Mabb. against *Staphylococcus aureus*

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Abstract

The leaves of *Dracaena trifasciata* (Prain) Mabb. are known to have medicinal properties, including antibacterial activity. Among the bacteria of concern, *Staphylococcus aureus* poses a significant challenge due to its frequent resistance to antibiotics. This study was driven by the growing urgency to address the resistance of bacteria through the exploration of alternative antimicrobial agents derived from natural products such as *D. trifasciata*. The objectives were to identify the active compounds with antibacterial activity in the potential extracts, fractions and isolates of *D. trifasciata* against *S. aureus* and observe the structural changes in bacterial cells after exposure to the leaf fractions of the plant. Compound identification was performed using liquid chromatography-high resolution mass spectrometry (LC-HRMS), while compound separation was conducted through thin layer chromatography (TLC) and preparative TLC. Scanning electron microscopy (SEM) was used to observe the effects of fraction treatment on bacterial cells. The disc diffusion method was used for antibacterial testing, with inhibition zone parameters. The results showed that the leaf extract of *D. trifasciata* exhibited significant antibacterial activity against *S. aureus*, as evidenced by significant inhibition zones and SEM-based detection of structural damage to bacterial cells. Identified active compounds included hexamethylenetetramine, reserpine, (2E)-3-(4-hydroxyphenyl)-N-[2-(4-hydroxyphenyl)ethyl]acrylamide, 2-[(3S)-1-(cyclohexylmethyl)-3-pyrrolidinyl]-1H-benzimidazole-5-carbonitrile, palmitic acid, adenosine, acridine-9(10H)-thione, galaxolidone and 3,5-di-tert-butyl-4-hydroxybenzoic acid. Antibacterial research on *D. trifasciata* was carried out from crude extract to separate fractions using the bioassay test principle and showed the potential of this plant as a natural antibacterial source.

Keywords: active compound; antibacterial; *Dracaena*; *Staphylococcus aureus*

Introduction

Dracaena trifasciata (Prain) Mabb., or *Sansevieria trifasciata* Prain, belongs to the Asparagaceae family. This perennial herb, native to tropical West Africa, is often cultivated as an ornamental plant in homes, gardens and landscapes around the world (1). *D. trifasciata*, also known as snake plant (mother-in-laws' tongue), has an ethnobotanical history. Its phytochemical compounds, such as alkaloids, tannins, terpenoids, saponins, flavonoids, steroids and phenols, play a role in inhibiting the growth of various pathogenic bacteria, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi*, *Bacillus subtilis* and *Klebsiella pneumoniae* (2–5). Recent studies have reported that *D. trifasciata* leaf extract has significant antibacterial effects and has the potential to be a solution to antibiotic resistance (6–10). *S. aureus* infection is one of the most common bacterial infections. This bacterium is a major causative agent of various nosocomial infections and diseases, including endocarditis, osteomyelitis, bacteremia, pneumonia, skin infections and complaints related to implanted devices (11–15). Despite the availability of various antibiotics within healthcare systems, the threat of fatal infections has neither diminished nor stabilised in both animals and humans. The misuse of antibiotics has led to a concerning risk of bacterial resistance. Currently approved antibiotics, such as penicillin and tetracycline, are ineffective against *S. aureus* infections, which can be attributed to significant resistance. Therefore, it is crucial to develop new and effective antibacterial agents to combat invasions caused by the strains of bacteria (11, 16).

Phytochemistry is a vital branch of modern therapeutic study due to the availability, low toxicity, chemical diversity and biochemical specificity of natural compounds (17, 18). At present, medicinal plants are considered a valuable alternative to synthetic chemical medications in pursuit of environmental health. A primary advantage of these alternatives is the reduced side effects compared to synthetic drugs (19–23). Furthermore, investigations on herbal products have significantly advanced the fields of medicine and pharmacology. The ability to extract and isolate a wide range of phytochemicals from various plants remains a driving force behind global efforts to discover new bioactive compounds and their pharmacological properties (24–27). The emerging trend in antibiotic-resistant bacteria presents an emerging public health threat, necessitating an immediate prospect for alternative therapeutic agents. The prospect of alternative therapeutic agents presents an uphill struggle in the advent of increasing resistance to antibiotics. *S. aureus* is one of the pathogenic bacteria to develop resistance to a variety of antibiotics. Among all pathogenic bacteria, *S. aureus* has been most notorious in having developed resistance to a series of antibiotics (24). Hence, this condition requires an exploration of natural products, which can offer strong antibacterial activities. Therefore, in this context, leaves of *D. trifasciata*, one of the most used medicinal plants, have come to prominence as they can offer antibacterial activities (10, 28, 29).

Previously reported studies on the antibacterial potential of *D. trifasciata* have not yet achieved complete identification and characterisation. This study aims to identify active antibacterial compounds of the extract of this plant and find out their effect on bacterial cell structure. The cutting-edge analytical techniques, i.e., LC-HRMS and SEM, were utilised in order to achieve this goal. The technique of bioassay-guided fractionation was used in order to trace this antibacterial characteristic from the crude extract to the purified.

Materials and Methods

The *Dracaena trifasciata* leaves used in this study were obtained from previous research. Samples were collected between January and March 2024 on the island of Java, Indonesia. The ethanol extract was prepared through a multistage sonication-assisted extraction, employing chloroform followed by ethanol as solvents (30). *Staphylococcus aureus* ATCC 25923 was used as the test bacterium. Extraction and fractionation were also carried out at Bio-techno Industry Laboratory, Universitas Atma Jaya Yogyakarta. Antibacterial assay and SEM were also carried out at the Integrated Research and Testing Laboratory (LPPT) Universitas Gadjah Mada (UGM). The identification of the compound was also carried out in the Integrated Research Laboratory of Brawijaya University (UB).

Fractionation using vacuum liquid chromatography and thin-layer chromatography

The ethanol extract of *D. trifasciata* leaves was fractionated using vacuum liquid chromatography (VLC). In the process, a total of 2.5 g of extract was mixed with 5 g of silica gel 60 GF_{254} powder. The packing of the stationary phase was performed by gradually adding 12 g of silica gel GF_{254} powder into a sintered glass funnel. During the packing process, the vacuum was continuously maintained to achieve a compact and solid stationary phase approximately 2 cm high. The mixture of *D. trifasciata* leaf extract and silica gel was then placed into the sintered glass (31, 32).

The type of eluents used as the mobile phase was selected based on the polarity of the solutions and extracts, specifically n-hexane: chloroform (3:1, 2:2, 1:3 v/v), 100 % chloroform and chloroform: ethanol (3:1, 2:2, 1:3 v/v), as well as 100 % ethanol. Furthermore, the total volume of the mobile phase mixture was 100 mL. The eluent was poured slowly under a vacuum to ensure uninterrupted elution. Fractions collected were stored in porcelain dishes, covered with aluminium foil perforated for ventilation. These were allowed to dry in a maceration cabinet for 2–3 days until a paste formed, after which the fractions were stored in 10 mL flask bottles and refrigerated to preserve phytochemical integrity (33). The resulting paste from the fractionation was analysed for its TLC profile. Silica gel F_{254} served as the stationary phase, while an ethyl acetate: methanol mixture in a 3:2 (v/v) ratio was the mobile phase. The TLC results were observed under UV light at wavelengths of 254 and 366 nm. Finally, fractions showing similar spot patterns were combined (34).

Fractionation of potential fractions of *Dracaena trifasciata* using preparative TLC

Ethanolic extract fraction was further refined through preparative TLC analysis. The fraction was applied to silica gel 60 GF254 (Merck) and developed with a 3:2 mixture of ethyl acetate and methanol (v/v). The separated bands were carefully scratched from the silica gel plates and the compounds were extracted with ethanol, which provides efficient recovery without significant dissolution of the silica matrix. The extracts were subsequently centrifuged and filtered to remove any possible silica particles before further analysis. Subsequently, the fractions were evaluated for antibacterial activity using a disc diffusion assay (31, 32, 35, 36).

Antibacterial activity analysis

Antibacterial activity assay on the fractions was carried out using the disc diffusion method. On the agar medium, 1 mL of bacterial liquid culture was inoculated using the pouring technique. The disc was dripped with 7.5 µL of the test sample. Negative control using distilled water and chloramphenicol (25 µg/disc) as a positive control. Bacterial cultures were incubated for 24 hr at 37 °C. The diameter of the clear zone was used as a parameter for antibacterial activity was analysed by measuring the diameter of the clear zone around the discs (37).

SEM analysis

SEM was performed to observe morphological changes in the cell wall and morphology of *S. aureus* in the control and treated groups (38, 39). Untreated *S. aureus* and *S. aureus* treated with the most active fraction of *D. trifasciata* ethanolic extract were incubated at 37 °C for 24 hr. Following incubation, each tube containing cultures was centrifuged at 4000 × g for 10 min at 4 °C. The samples were rinsed twice in distilled water and resuspended in 1 mL of water. Subsequently, 10 µL of the suspension was applied to the 1 × 1 cm slide. Samples were 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 using a sputter coater (Quorum Technologies Ltd.) for 10 min. SEM imaging was then performed using a Quanta 650 Field Emission SEM (FEI) (40).

LC-HRMS analysis

Screening for bioactive compounds was conducted on the ethanol extract, selected fractions and isolates derived from the leaves of *D. trifasciata*, which had the best antibacterial activity. Liquid chromatographic separation was performed at the Central Laboratory for Life Sciences (Laboratorium Sentral Ilmu Hayati, LSIH), Brawijaya University, using a Hypersil GOLD aQ column (50 × 1 mm, 1.9 µm particle size) with an injection volume of 100 µL. The mobile phases consisted of solvent A (0.1 % formic acid in water) and solvent B (0.1 % formic acid in acetonitrile), delivered at a flow rate of 40 µL/min. The chromatographic run was maintained for 70 min. Eluted compounds were subsequently analysed by mass spectrometry on a thermo scientific Q exactive instrument equipped with an electrospray ionisation (ESI) source operating in positive ion mode. Instrument parameters were set as follows: sheath gas (N_2) pressure, 50 psi; spray voltage, 4.5 kV; capillary temperature, 300 K; and mass scan range, m/z 50–750. Compound detection and annotation were carried out using Compound Discoverer software in conjunction with the mzCloud MS/MS spectral library (41).

Data analysis

All experiments were conducted in triplicate and the results obtained were analysed both qualitatively and quantitatively. The antibacterial assay was analysed quantitatively, while data were processed and analysed using SPSS 26 (42) and one-way ANOVA, respectively, followed by post-hoc analysis with Tukeys' test at a 95 % confidence level. The identification of bioactive compounds from LC-HRMS analysis was presented descriptively.

Results

Antibacterial activity of TLC-separated fractions

The potential extract obtained from the study conducted was separated using VLC, leading to 6 fractions. Silica gel 60 GF254 powder functions as the stationary phase in the separation process. Owing to its highly porous and polar nature, silica gel interacts with extract compounds according to their polarity. Non-polar compounds are eluted more rapidly by the mobile phase (solvent), whereas polar compounds are retained longer. Prior to fractionation using VLC, silica gel is mixed with the extract to enable its application onto the column as a homogeneous dry powder. This procedure enhances separation efficiency, minimises tailing and optimises compound interaction with the stationary phase. The antibacterial activity of the fractions obtained from the TLC separation was evaluated to determine the effectiveness against bacterial strains. Furthermore, each fraction was tested for its ability to inhibit bacterial growth. The results were presented for the specific fractions analysed. Analysis was conducted using TLC to combine fractions with similar patterns, generating a final three, as presented in Fig. 1.

The three fractions tested showed significantly different antibacterial activity. Fraction 3 had an average inhibition zone of 12.33 ± 0.58 mm, which is categorised as strong. This activity was the strongest compared to the other two fractions. This indicates that fraction 3 contains bioactive compounds that are effective against the tested bacterial strains. Fractions 1 and 2, which had moderate antibacterial activity, showed a different result (Fig. 2, Table 1). Differences in bioactive compound content can affect their effectiveness. The presence of specific phytochemicals in fraction 3 could be responsible for its enhanced potency, potentially offering a basis for further isolation and characterisation of the compounds.

Previous research also showed that fractionated plant extracts have diverse antibacterial properties. Fractionation, conducted to simplify the composition of bioactive components in the extract, is important for identifying effective antimicrobial agents. Furthermore, research is expected to focus on further analysis of fraction 3 to determine specific compounds that act as potent antibacterials. Exploration of the mechanisms of action of specific compounds against pathogenic bacteria is also necessary. Guided by this bioactivity, only fraction 3 was selected for further purification using preparative TLC. As shown in Table 2, this separation produced four sub-fractions with varying levels of antibacterial activity.

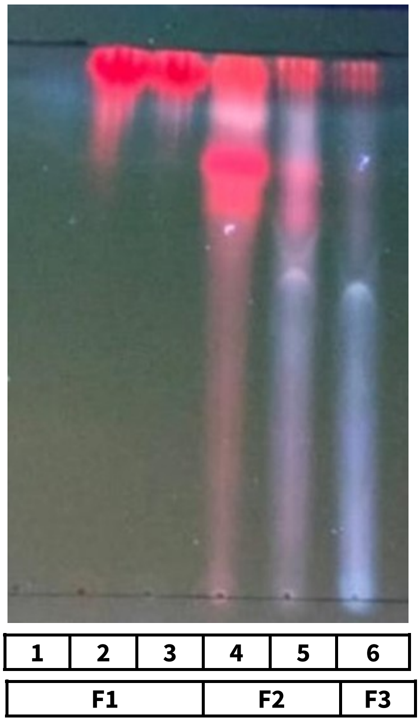


Fig. 1. Results of extract separation by thin layer chromatography (TLC). F1: fraction 1; F2: fraction 2; F3: fraction 3.

Table 1. Antibacterial activity of a potential fraction of *D. trifasciata* leaves against *S. aureus*

Sample	**Clear zone (mm)**
Control (+) Aquadest/distilled water	0.00 ± 0.00^{a}
Control (-) Chloramphenicol	24.67 ± 0.58^{d}
Fraction 1	8.33 ± 0.58^{b}
Fraction 2	9.33 ± 0.58^{b}
Fraction 3	12.33 ± 0.58^{c}

*The antibacterial activity values are expressed as mean ± standard error from three replicates. Different superscript letters indicate significant differences at ($p < 0.05$) according to the Tukey test

Antibacterial activity testing of TLC-preparative fractions

Fraction 3, which showed the highest potential as an antibacterial agent, was further separated using preparative TLC, leading to four potential fractions (Fig. 3). The antibacterial activity of these fractions was tested, with isolate 1 classified as strong (18.33 ± 0.58 mm) (Fig. 4, Table 2). This activity was significantly different compared to isolates 2, 3 and 4. A stronger activity was observed when isolated

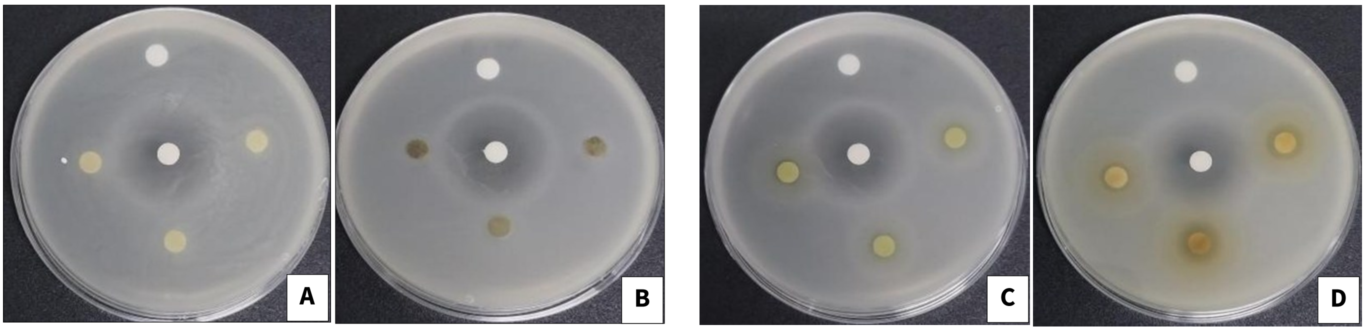


Fig. 2. Antibacterial activity of *Dracaena trifasciata* fractions against *Staphylococcus aureus*.

(A) Negative control (aquadest/distilled water) and positive control (chloramphenicol); (B) fraction 1; (C) fraction 2; (D) fraction 3.

Table 2. Antibacterial activity of four potential fractions of preparative TLC fractionation results from fraction 3

Sample	Inhibition zone (mm)
Control (+) Chloramphenicol	24.67±0.58^{d}
Control (-) Aquadest/distilled water	0.00±0.00^{a}
Potential fraction 1	18.33±0.58^{d}
Potential fraction 2	8.83±0.76^{c}
Potential fraction 3	3.66±1.58^{b}
Potential fraction 4	2.33±0.58^{b}

*The antibacterial activity values are expressed as mean ± standard error from three replicates. Different superscript letters indicate significant differences at ($p < 0.05$) according to the Tukey test.

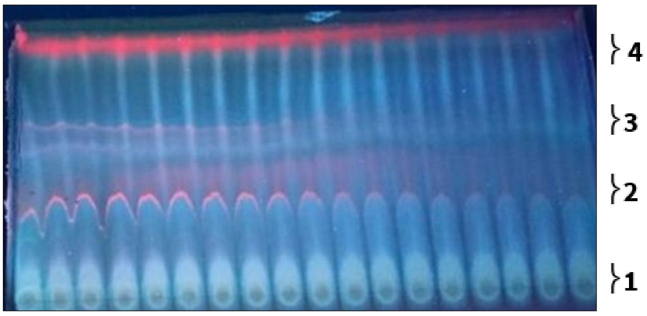


Fig. 3. Results of preparative TLC with four layers of fractions observed under UV 366.

(1) fraction 1; (2) fraction 2; (3) fraction 3; (4) fraction 4

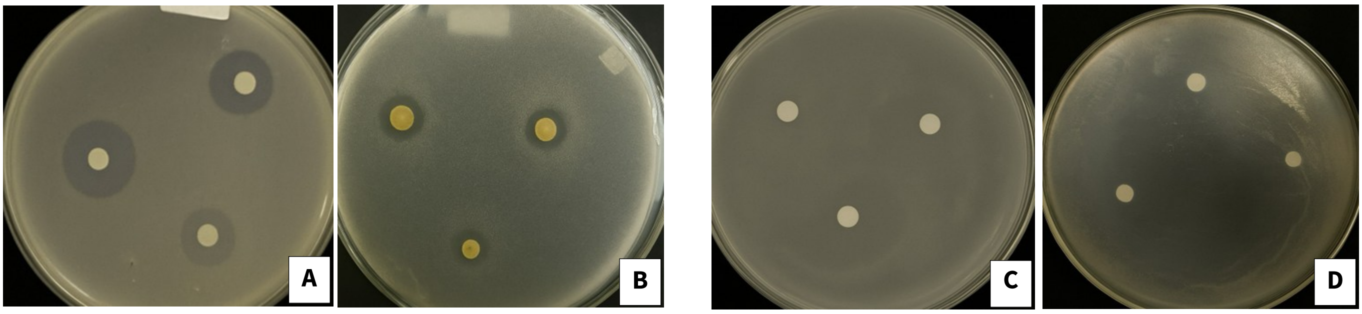


Fig. 4. The antibacterial activity of fractions leaves of *Dracaena trifasciata* ethanolic extract against *Staphylococcus aureus*.

(A) potential fraction 1; (B) potential fraction 2; (C) potential fraction 3; (D) potential fraction 4.

potential fractions were exposed to *S. aureus*. This is attributed to the fact that compounds isolated from TLC preparative were more specific and played a significant role in damaging bacterial cells. The reduced activity observed in potential fractions 2–4 suggests that the antibacterial compounds were concentrated primarily in potential fraction 1. This progressive increase in activity after sub-fractionation strongly supports the efficiency of the bioassay-guided isolation strategy, whereby successive purification steps allow localisation and enrichment of the true bioactive constituents.

Observation of bacterial cell damage by SEM

Observation with SEM was conducted to study the pattern of changes in the morphology and structure of bacterial cells under the influence of the potential fraction of *D. trifasciata* ethanolic extract. The changes were observed in general appearance, cell size and damage to cytoplasmic walls and membranes. Bacterial cells exposed to the potential fraction from *D. trifasciata* leaves showed evidence of bacterial damage, as presented in Table 3. Compared to untreated controls, treated cells showed evident structural damage, possibly due to the active compounds in the extract, which may compromise the integrity of the bacterial cell.

Identification of active compounds

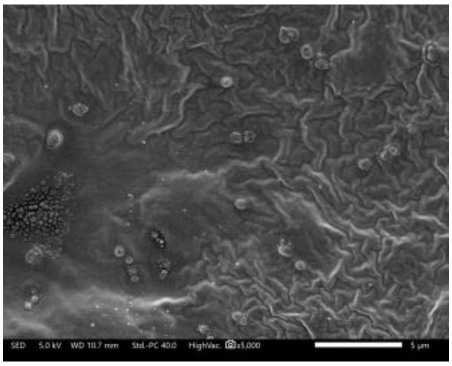
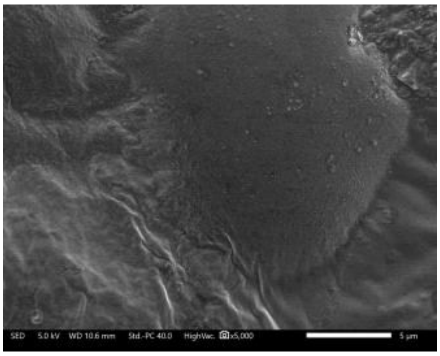
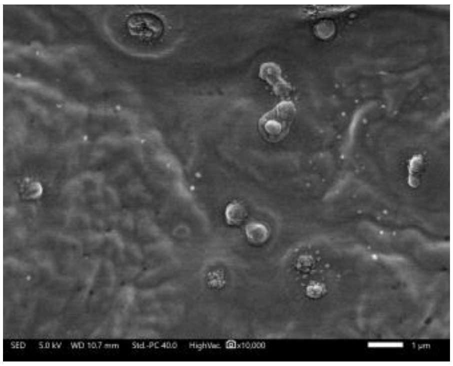
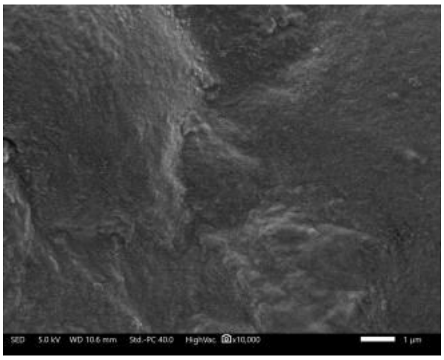
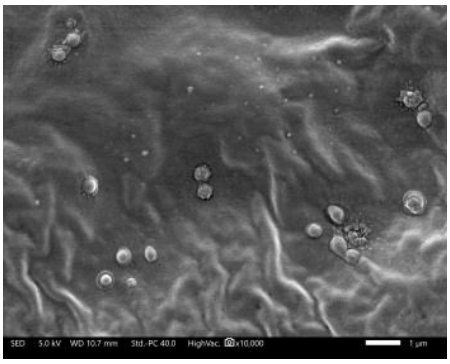
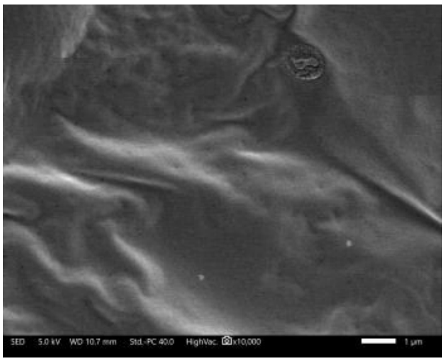
LC-HRMS analysis was conducted on the extract, bioactive fractions and the most potent isolate to identify the compounds responsible for antibacterial activity. The extract predominantly contained 4-coumaric acid, catechin, 2-hydroxycinnamic acid, ouabain, 4-methoxycinnamic acid and 3-acetyl-11-keto-β-boswellic acid (Fig. 5, Table 4). In contrast, the fractions were rich in α-eleostearic acid, α-linolenic acid, isoleucine, tyramine, kaempferol and acridine-9(10H)-thione, as shown in Fig. 6 and Table 5. The isolates primarily contained palmitic acid, nicotinamide, reserpine, ostruthin, betaine and diisobutyl phthalate, as detailed in Fig. 7 and Table 6. Compounds that were consistently present in the extract, fractions and potential isolates included hexamethylenetetramine, reserpine, (2E)-3-(4-hydroxyphenyl)-N-[2-(4-hydroxyphenyl)ethyl]acrylamide, 2-[(3S)-1-(cyclohexylmethyl)-3-pyrrolidinyl]-1H-benzimidazole 5-carbonitrile, palmitic acid, adenosine, acridine-9(10H)-thione, galaxolidone and 3,5-di-tert-butyl-4-hydroxybenzoic acid.

Discussion

The antibacterial activity of fractions obtained from TLC separation emphasised the significance of fractionation in isolating bioactive compounds with potential therapeutic effects. While the methanolic extract of *D. trifasciata* leaves showed good inhibition of 15 mm against *S. aureus* (43). The ethanolic extract in this study produced a stronger effect, reaching 18.33 mm (30). This study confirmed variations in antibacterial activity among the fractions against *S. aureus*. Fraction 3 demonstrated the highest antibacterial activity, with an average inhibition zone diameter of 12.33 ± 0.58 mm, categorising it as strong. The structure of bioactive compounds holding potential to inhibit *S. aureus* must undergo increased research into their mechanism of action. On the other hand, fractions 1 and 2 showed moderate activity, whose rationale could lie in terms of varied concentrations and classes of phytochemicals. The latter validates the complexity of plant extracts and the significance of in-depth fractionation to define and isolate active antimicrobial entities. The results concur with previous studies showing vast differences in antibacterial activities in various fractions of plant extracts, in support of in-depth research in this regard (30).

The phytochemical composition and the antibacterial and antioxidant activities of *D. trifasciata* leaves have been described at the crude extract level, but failed to clearly identify certain active fractions or bioactive molecules responsible for the aforementioned antibacterial activities. The present study addresses this lacuna through an application of the bioassay-guided isolation (BGI) approach. Successive fractionation and activity-guided assay resulted in the identification of the most active fractions and sub-fractions, whose identities were established through LC-HRMS analysis for identification of compounds. Hence, this research not

Table 3. Comparison of bacterial cells treated with *Dracaena trifasciata* extract to control

Magnification (x)	**Treatment**	
	Control	***S. aureus* treated with potential fraction isolate of *D. trifasciata* leaves**
5.000		
10.000		
10.000		

Scanning electron microscopy (SEM) was adopted to examine morphological and structural changes in bacterial cells influenced by the potential fraction (fraction 1) of *Dracaena trifasciata* ethanolic extract. The analysis focused on identifying changes such as overall appearance, cell size and damage to the cytoplasmic wall and membrane

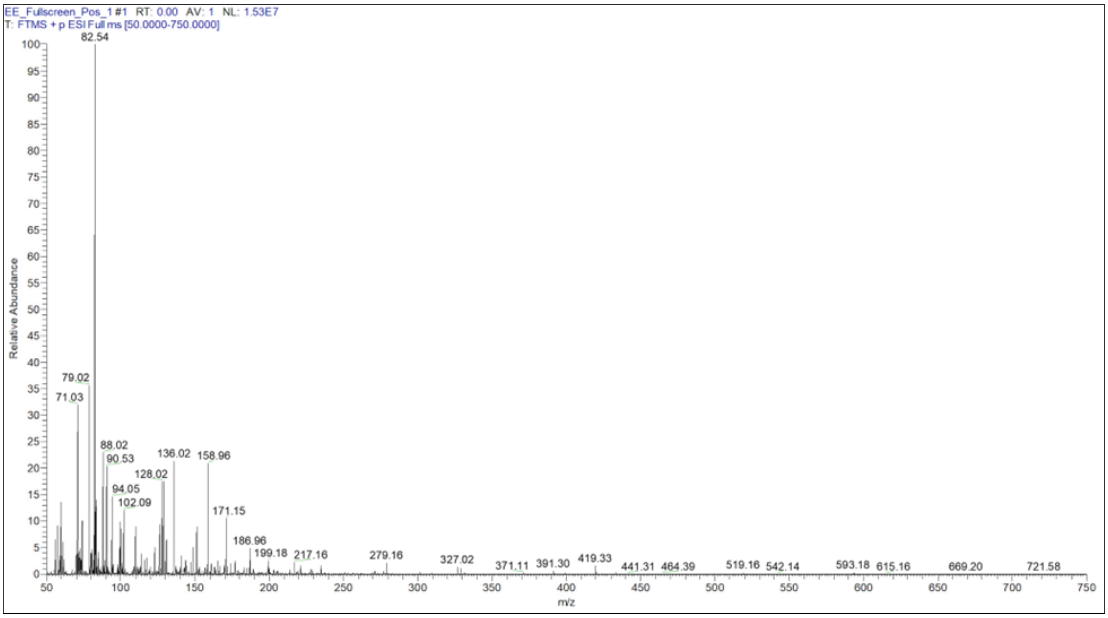


Fig. 5. LC-HRMS chromatogram of chemical components Leaves of *Dracaena trifasciata* ethanolic extract.

Table 4. Results of identification of active compound from ethanolic extract of *Dracaena trifasciata* leaves using LC-HRMS

Name	Formula	Molecular weight	RT [min]	Area (Max.)	mzCloud best match
Choline	$C_5H_{13}NO$	103.09998	0.857	5476721556.25	97.8
NP-003037	$C_{16}H_{22}O_8$	380.08425	0.838	894699698.70	98.4
(7E,13E)-9,15-dihydroxy-4,10,16-trimethyl-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione	$C_{16}H_{22}O_8$	382.12055	0.847	492346626.35	87.4
NP-019811	$C_6H_7NO_2$	125.04788	0.899	335906648.22	93.1
Hexamethylenetetramine	$C_6H_{12}N_4$	140.10608	26.397	312944951.72	98.6
Isoleucine	$C_6H_{13}NO_2$	131.09468	1.281	237509223.80	99.5
α-Eleostearic acid	$C_{18}H_{30}O_2$	278.23003	16.653	169619414.15	95.8
(2E)-3-(4-Hydroxyphenyl)-N-[2-(4-hydroxyphenyl)ethyl]acrylamide	$C_{17}H_{17}NO_3$	283.12632	8.632	144011517.10	98.1
Nicotinamide	$C_6H_6N_2O$	122.04818	0.909	125326362.54	83.7
Cafestol	$C_{20}H_{28}O_3$	294.22755	17.368	112225542.35	91.1
Reserpine	$C_{33}H_{40}N_2O_9$	608.28484	21.017	100769230.87	74.7
Caprolactam	$C_6H_{11}NO$	113.08429	3.051	96516527.77	98.8
4-Coumaric acid	$C_9H_8O_3$	164.0474	6.437	92348015.33	99.1
Acetylcholine	$C_7H_{15}NO_2$	113.08409	0.904	83630321.06	67.1
Zearalenone	$C_{18}H_{22}O_5$	278.15864	17.815	83395715.40	89
NP-007909	$C_{13}H_{20}O_3$	206.13114	5.368	80201485.59	65.7
Adenine	$C_5H_5N_5$	135.05451	0.895	78314558.57	75.4
11-α-Hydroxy-17-methyltestosterone	$C_{20}H_{30}O_3$	318.22545	16.644	75993937.12	79.3
4-Guanidinobutyric acid	$C_5H_{11}N_3O_2$	145.0851	0.904	73339894.04	74.3
Nicotinic acid	$C_6H_5NO_2$	123.03217	0.91	70523065.12	84.6
Catechin	$C_{15}H_{14}O_6$	290.08223	0.879	69045080.97	61.2
L-Phenylalanine	$C_9H_{11}NO_2$	165.07898	1.792	68048230.52	99.4
1-Aminocyclohexanecarboxylic acid	$C_7H_{13}NO_2$	143.09462	0.897	61922027.67	78.9
2-(Cyclohexylmethylidene)-1,2,3,4-tetrahydronaphthalen-1-one	$C_{17}H_{20}O$	240.14966	0.91	60316290.55	84.4
2-[(3S)-1-(Cyclohexylmethyl)-3-pyrrolidinyl]-1H-benzimidazole-5-carbonitrile	$C_{19}H_{24}N_4$	308.20345	18.795	58422229.85	92.6
L-Tyrosine	$C_9H_{11}NO_3$	164.04739	0.909	54620836.74	75.7
N-({(2R,4S,5R)-5-[3-(2-Methoxyphenyl)-1-methyl-1H-pyrazol-5-yl]-1-azabicyclo[2.2.2]oct-2-yl}methyl)acetamide	$C_{21}H_{28}N_4O_2$	350.21694	10.311	44950668.21	71.2
2,4-dihydroxy-6-methyl-3-[(2E)-3-methyl-5-[(1S,2R,6R)-1,2,6-trimethyl-3-oxocyclohexyl]pent-2-en-1-yl]benzaldehyde	$C_{23}H_{32}O_4$	394.21095	7.616	43726902.09	97.3
3-hydroxy-3-methylpentanedioic acid	$C_6H_{10}O_5$	184.03482	2.06	35561437.43	77.2
Thromboxane B2	$C_{20}H_{34}O_6$	408.19042	5.169	34858243.85	86.8
Trigonelline	$C_7H_7NO_2$	137.04761	0.869	33106616.78	96.7
2,5,6,8-Tetramethyl-4,5,6,7,8,9-hexahydropyrimido[5',4' 5,6]pyrido[3,4-d][1,3]oxazole-4,7,9-trione	$C_{12}H_{12}N_4O_4$	276.08745	0.852	32033052.76	86.3
5-(6-hydroxy-6-methyloctyl)-2,5-dihydrofuran-2-one	$C_{13}H_{22}O_3$	208.14699	6.108	30539170.11	81.5
QLH	$C_{17}H_{28}N_6O_5$	418.19609	6.975	30262167.33	64
D-Carnitine	$C_7H_{15}NO_3$	161.10512	0.879	30097881.91	77.6
D-Glucosamine	$C_6H_{13}NO_5$	179.07932	0.844	29940546.10	87.2
Decarbamoyl-neosaxitoxin	$C_9H_{16}N_6O_4$	272.12798	5.352	29107328.03	93.8
1-[3-Methyl-5-(2-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl]ethan-1-one	$C_{18}H_{15}N_3O_3$	299.12397	6.851	28359041.75	87.6
4-Fluoro furanyl fentanyl 3-furancarboxamide	$C_{24}H_{25}FN_2O_2$	392.1935	7.597	28208365.49	67.4
N-Butylbenzenesulfonamide	$C_{10}H_{15}NO_2S$	213.08295	26.986	27399858.40	85.6
Sedanolide	$C_{12}H_{18}O_2$	194.13068	10.921	26624191.96	60.3
NP-018660	$C_{13}H_{22}O_3$	208.1467	7.255	26624095.69	75.9

Palmitic Acid	$C_{16} H_{32} O_2$	273.27117	14.272	26470496.23	84.5
6-[(3R,4S,5S,7R)-7-[(2S,3S,5S)-5-ethyl-5-[(2R,5R,6S)-5-ethyl-5-hydroxy-6-methyloxan-2-yl]-3-methyloxolan-2-yl]-4-hydroxy-3,5-dimethyl-6-oxononyl]-2-hydroxy-3-methylbenzoic acid	$C_{34} H_{54} O_8$	628.34386	9.972	25675102.95	78.7
15-Deoxy-Δ12,14-prostaglandin J2-2-glycerol ester	$C_{23} H_{34} O_5$	412.22185	5.917	25197542.50	98
(2R,3R,4S,5S,6R)-3-(acetyloxy)-2-[(acetyloxy) methyl]-5-(hexanoyloxy)-6-[(2S,3R)-2,3,4-trihydroxybutoxy]oxan-4-yl tetradecanoate	$C_{34} H_{60} O_{13}$	698.36943	14.599	25122860.22	91.4
NP-006888	$C_{13} H_{24} O_4$	226.15837	6.717	22368909.27	66.3
NP-014175	$C_{20} H_{28} O_3$	676.37162	16.342	22204371.85	67
Adenosine	$C_{10} H_{13} N_5 O_4$	267.10106	1.181	21727904.89	99.6
Acepromazine	$C_{19} H_{22} N_2 O S$	326.14253	10.314	21672026.13	84.2
Sakuranetin	$C_{16} H_{14} O_5$	286.08956	11.767	21167407.73	79.9
(2R,3R,3aR,5R)-5,7-dimethoxy-2-(7-methoxy-2H-1,3-benzodioxol-5-yl)-3-methyl-3a-(prop-2-en-1-yl)-2,3,3a,4,5,6-hexahydro-1-benzofuran-6-one	$C_{22} H_{26} O_7$	424.14916	5.116	19925565.10	93.5
(1R,4aS)-7-(2-Hydroxypropan-2-yl)-1,4a-dimethyl-9-oxo-3,4,10,10a-tetrahydro-2H-phenanthrene-1-carboxylic acid	$C_{20} H_{26} O_4$	330.18901	12.379	19645776.41	80.3
Isobutyl sildenafil	$C_{23} H_{32} N_6 O_4 S$	976.43772	12.505	19150844.01	61.3
2-Hydroxycinnamic acid	$C_9 H_8 O_3$	146.03681	6.711	18986162.15	61.3
NP-008952	$C_{12} H_{20} O_4$	250.12137	10.136	17890561.89	94.4
(1R,5S,6S)-3-(hydroxymethyl)-5-{[(2Z)-2-methylbut-2-enoyl]oxy}-2-oxo-6-(propan-2-yl)cyclohex-3-en-1-yl (2E)-4-hydroxy-2-methylbut-2-enoate	$C_{20} H_{28} O_7$	402.16423	4.676	17543815.16	89.6
1-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-8-methyl -1,2,3,4-tetrahydroquinoline	$C_{15} H_{18} N_4 O_2$	286.14473	7.383	17457204.38	92.6
methyl (1R,4aS,9S,10S)-10-(acetyloxy)-5,9-dihydroxy-1,4a-dimethyl-2-oxo-7-(propan-2-yl)-1,2,4a,9,10,10a-hexahydrophenanthrene-1-carboxylate	$C_{23} H_{28} O_7$	438.16701	6.122	17186307.17	96.8
N4-phenethylmorpholine-4-carbothioamide	$C_{13} H_{18} N_2 O S$	272.09157	1.231	16728614.59	97.3
[3,2-c]Pyrazole-androst-4-en-17β-ol	$C_{20} H_{28} N_2 O$	312.2159	11.163	16362179.96	69.6
NP-010966	$C_{23} H_{32} O_5$	410.20607	6.457	16262863.20	94.7
2-[(3R,4S)-1-Benzoyl-3-ethyl-4-piperidinyl]-N -(4-fluorobenzyl)acetamide	$C_{23} H_{27} F N_2 O_2$	404.18004	5.6	15856713.06	98.3
6-methyl-1-(2-methylphenyl)-3-propylfuro [3,2-d]pyrimidine-2,4(1H,3H)-dione	$C_{17} H_{18} N_2 O_3$	336.09078	0.852	15746895.93	78.6
4-(4-Chlorophenyl)-3-[3-(trifluoromethyl) phenyl]-2,3-dihydro-1,3-thiazol-2-imine	$C_{16} H_{10} Cl F_3 N_2 S$	354.01631	19.799	15577415.10	90
acridine-9(10H)-thione	$C_{13} H_9 N S$	211.04619	16.917	14536549.78	96.9
NP-017061	$C_{20} H_{30} O_4$	334.22057	14.871	14487476.56	83.6
Octyl decyl phthalate	$C_{26} H_{42} O_4$	440.29173	18.501	14478962.55	96
12-oxo Phytodienoic Acid	$C_{18} H_{28} O_3$	274.19872	13.857	14473439.32	64.2
Acetophenone	$C_8 H_8 O$	120.05779	1.298	14360449.80	78.8
4-Indolecarbaldehyde	$C_9 H_7 N O$	145.05274	7.593	14236625.92	93.2
9S,13R-12-Oxophytodienoic acid	$C_{18} H_{28} O_3$	292.20928	16.355	14214891.38	68.2
NP-017176	$C_{27} H_{40} O_5$	426.27571	10.223	14148287.17	81.7
NP-001846	$C_{11} H_{20} O_4$	238.12032	11.684	13798338.58	96.7
THJ	$C_{22} H_{22} N_4 O$	716.35001	15.514	12962411.65	72.8
4,6-dimethyl-2,7-diphenyl-3,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-one	$C_{20} H_{17} N_3 O$	315.1378	1.244	12665620.52	88.9
(3R,4R)-N-Ethyl-4-hydroxy-3-[(4-methoxybenzoyl)amino]-1-azepanecarboxamide	$C_{17} H_{25} N_3 O_4$	357.16738	14618	12594530.47	71.1
3'-Hydroxystanozolol	$C_{21} H_{32} N_2 O_2$	344.24285	19.113	12374689.66	96.2
methyl 2-cyano-3-{1-[3-(1H-imidazol-1-yl) propyl]-1H-pyrrol-2-yl}acrylate	$C_{15} H_{16} N_4 O_2$	306.11495	6.098	12083387.78	96.7
DL-α-Aminocaprylic acid	$C_8 H_{17} N O_2$	159.12592	1.339	12034899.77	83.1
4-Fluoro tetrahydrofuran fentanyl	$C_{24} H_{29} F N_2 O_2$	396.22652	7.099	11822031.30	76.3

1-(4-benzylpiperazino)-2-(pyridin-2-ylamino) propan-1-one	$C_{19}H_{24}N_4O$	692.3494	16.341	11777452.98	74.6
3-(2-chlorophenyl)-N-(2,3-dihydro-1H-inden-2-yl)-5-methylisoxazole-4-carboxamide	$C_{20}H_{17}ClN_2O_2$	330.12058	13.203	11745078.54	93.6
4-Hydroxynordiazepam	$C_{15}H_{11}ClN_2O_2$	286.05315	6.502	10692276.81	95.1
5-hydroxy-2,2,6,6-tetramethyl-4-{3-methyl-1-[2,4,6-trihydroxy-3-(2-methylpropanoyl) phenyl]butyl}cyclohex-4-ene-1,3-dione	$C_{25}H_{34}O_7$	428.21871	4.382	10684031.34	93.5
NP-021018	$C_{12}H_{18}O_4$	248.10568	9.725	10580529.60	94.1
Galaxolidone	$C_{18}H_{24}O_2$	272.18184	17.723	10224278.74	97.4
Ouabain	$C_{29}H_{44}O_{12}$	606.27098	20.217	9912080.33	83.9
NP-003117	$C_{13}H_{20}O_4$	262.12233	10.881	9906201.87	97.2
Nevirapine	$C_{15}H_{14}N_4O$	266.1173	8.583	9596928.82	96.8
4-(cyclohexylcarbonyl)-1-phenethylpiperazine-2,6-dione	$C_{19}H_{24}$ N2 O3	306.19175	13.293	8947490.81	68.5
2,2-Methylenebis(4-ethyl-6-tert-butylphenol)	$C_{25}H_{36}O_2$	390.25054	15.813	8474926.99	97.1
2-(1-benzothiophen-3-ylmethylene)-3,4-dihydronaphthalen-1(2H)-one	$C_{19}H_{14}OS$	290.08223	1.228	8398656.10	72
4-Methoxycinnamic acid	$C_{10}H_{10}O_3$	178.06297	20.503	8041839.91	96.8
(2R,3S,4S,5S,6R)-2-({7-hydroxy-4-[(3Z)-5-hydroxy-3-methylpent-3-en-1-yl]-4a,8,8-trimethyl-3-methylidene-decahydronaphthalen-2-yl}oxy)-6-(hydroxymethyl)oxane-3,4,5-triol	$C_{26}H_{44}O_8$	506.28475	15.365	8018716.15	76.1
3-Acetyl-11-keto-β-boswellic acid	$C_{32}H_{48}O_5$	512.35503	19.876	7995284.98	93.5
NP-008882	$C_{27}H_{48}$ O9	538.30431	21.484	7665241.47	65.3
Mazindol	$C_{16}H_{13}ClN_2O$	284.07393	11.808	7466352.39	69.2
3,5-di-tert-Butyl-4-hydroxybenzoic acid	$C_{15}H_{22}O_3$	250.16013	14.64	7191463.59	90.9
N~1~-(2,5-dimethoxyphenyl)-N~2~-(2-pyridin-2-ylethyl)ethanediamide	$C_{17}H_{19}N_3O_4$	329.13651	7.137	6762292.26	97.7
Prolylleucine	$C_{11}H_{20}N_2O_3$	228.14968	1.283	6719782.26	90.3
NP-000919	$C_{11}H_{18}O_4$	196.1099	11.895	6677525.27	63.5

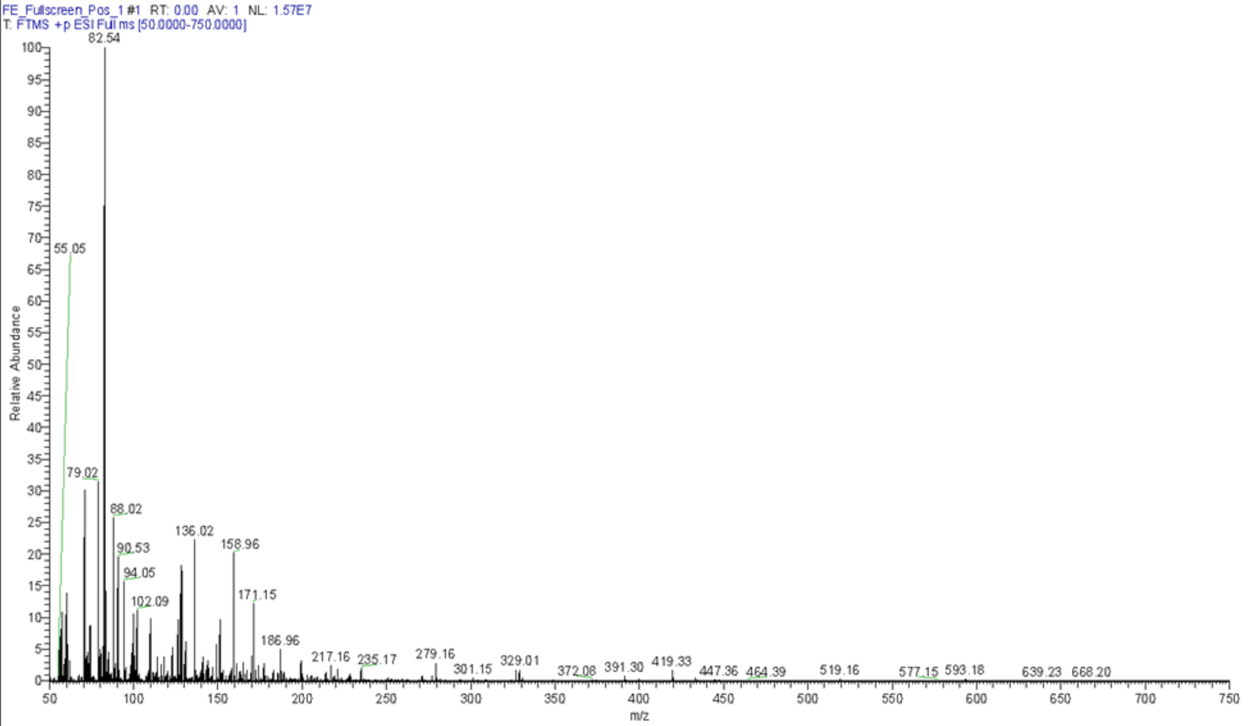


Fig. 6. LC-HRMS chromatogram of chemical components Leaves of *Dracaena trifasciata* potential fraction.

Table 5. Results of Identification of active compound from potential fractions of *Dracaena trifasciata* using LC-HRMS

Name	Formula	Molecular weight	RT [min]	Area (Max.)	mzCloud best match
L-Norleucine	$C_6H_{13}NO_2$	131.09467	1.285	461171248.37	99.8
2,5,6,8-Tetramethyl-4,5,6,7,8,9-hexahydropyrimido[5',4' 5,6] pyrido[3,4-d][1,3]oxazole-4,7,9-trione	$C_{12}H_{12}N_4O_4$	276.08742	0.846	391883396.28	94.9
Isoleucine	$C_6H_{13}NO_2$	131.09467	0.927	370648680.68	87.6
Hexamethylenetetramine	$C_6H_{12}N_4$	140.10593	26.398	298429734.00	98.6
5-Hydroxymethyl-2-furaldehyde	$C_6H_6O_3$	126.03181	0.866	297570843.59	76.6
4-Guanidinobutyric acid	$C_5H_{11}N_3O_2$	145.08513	0.917	233287155.96	85.6
Tyramine	$C_8H_{11}NO$	137.08416	0.933	203089843.84	70.7
3-(2,6-Dioxocyclohexyl)propanenitrile	$C_9H_{11}NO_2$	165.07892	0.928	152697327.37	63.3
L-Valine	$C_5H_{11}NO_2$	117.07921	0.925	139959529.39	97.6
methyl 2-(1,3,5-trihydroxy-4a-methyl-8-oxo-decahydronaphthalen-2-yl)prop-2-enoate	$C_{15}H_{22}O_6$	149.06728	0.859	139252711.61	98.8
DL-Leucineamide	$C_6H_{14}N_2O$	113.08416	0.886	100110616.43	86.1
L-Phenylalanine	$C_9H_{11}NO_2$	165.07892	1.8	82708664.57	99.5
2-[(3S)-1-(Cyclohexylmethyl)-3-pyrrolidinyl]-1H-benzimidazole-5-carbonitrile	$C_{19}H_{24}N_4$	308.20293	18.813	67324527.27	92.7
Kaempferol	$C_{15}H_{10}O_6$	286.05312	6.487	66104303.37	99.6
Pyridoxine	$C_8H_{11}NO_3$	169.07384	0.894	53292767.66	80.4
Adenine	$C_5H_5N_5$	135.05449	0.902	51833721.37	73.9
2-[(2-chlorobenzyl)sulfanyl]-4,6-dimethylnicotinonitrile	$C_{15}H_{13}ClN_2S$	326.00896	6.474	49691280.84	97.5
Zearalenone	$C_{18}H_{22}O_5$	278.15848	17.819	47092866.16	88.6
D-(+)-Glucose	$C_6H_{12}O_6$	197.08988	0.841	44277333.85	85.7
Vatalanib dihydrochloride	$C_{20}H_{15}ClN_4$	324.11561	0.861	42752836.13	98
(2,6-dimethylmorpholino)(1-methyl-5-nitro-1H-pyrazol-4-yl) methanone	$C11H_{16}N_4O_4$	258.07692	0.869	42613590.57	84.2
[3,2-c]Pyrazole-androst-4-en-17β-ol	$C_{20}H_{28}N_2O$	312.21568	11.17	38218267.82	72.7
Palmitic Acid	$C_{16}H_{32}O_2$	273.27098	14.288	35198246.12	83.5
pentane-1,2,3,4,5-pentol	$C_5H_{12}O_5$	174.05034	0.84	32001905.98	86.5
Acetophenone	C_8H_8O	120.05764	1.296	30494680.31	79.1
N-(2,5-diethoxy-4-morpholinophenyl)-4-methoxybenzenesulfonamide	$C_{21}H_{28}N_2O_6S$	414.19002	0.92	30233705.38	68.5
Reserpine	$C_{33}H_{40}N_2O_9$	608.28411	21.025	27441660.41	76.1
3-(1H-benzo[d]imidazol-2-yl)-6-ethyl-7-hydroxy-4H-chromen-4-one	$C_{18}H_{14}N_2O_3$	306.09935	0.844	27270916.01	93.1
N-(2,4-Dimethylphenyl)formamide	$C_9H_{11}NO$	149.084	0.936	27103094.73	81.4
Diisobutylphthalate	$C_{16}H_{22}O_4$	278.15706	17.823	26021900.59	99.1
QLH	$C_{17}H_{28}N_6O_5$	418.19585	6.988	24275450.79	61.6
2-Hydroxyphenylalanine	$C_9H_{11}NO_3$	181.07381	0.926	24029163.74	80.6
5-hydroxy-2,2,6,6-tetramethyl-4-{3-methyl-1-[2,4,6-trihydroxy-3-(2-methylpropanoyl)phenyl]butyl}cyclohex-4-ene-1,3-dione	$C_{25}H_{34}O_7$	428.21849	4.374	21290285.54	93.9
1-(4-benzylpiperazino)-2-(pyridin-2-ylamino)propan-1-one	$C_{19}H_{24}N_4O$	692.34922	16.356	20629051.70	75.2
β-Hydroxythiofentanyl	$C_{20}H_{26}N_2O_2S$	716.35001	15.528	17737198.65	69
4,6-dimethyl-2,7-diphenyl-3,7-dihydro-2H-pyrazolo[3,4-b] pyridin-3-one	$C_{20}H_{17}N_3O$	315.13744	1.243	17181459.78	89.4
T-2 Triol	$C_{20}H_{30}O_7$	404.17977	5.445	16953401.11	95.9
(3R,4R)-N-Ethyl-4-hydroxy-3-[(4-methoxybenzoyl)amino]-1-azepanecarboxamide	$C_{17}H_{25}N_3O_4$	714.33408	14.627	16597687.27	71.5
2-{[((2-hydroxyphenyl){[(2-hydroxyphenyl)methylene] amino}methyl)imino]methyl}phenol	$C_{21}H_{18}N_2O_3$	346.13387	4.351	16415121.18	69.7
NP-007909	$C_{13}H_{20}O_3$	206.13129	5.366	15742606.69	63.8
Adenosine	$C_{10}H_{13}N_5O_4$	267.10094	1191	15183584.43	99.6
α-Eleostearic acid	$C_{18}H_{30}O_2$	278.22984	16.65	14902234.15	91.7
NP-008882	$C_{27}H_{48}O_9$	538.30376	21.958	13794521.73	66.5
NP-010770	$C_{33}H_{40}N_2O_9$	608.28411	20.532	13306769.79	81.9
NP-007970	$C_{29}H_{42}O_9$	556.27129	6.923	12659065.99	93.6
YQH	$C_{20}H_{26}N_6O_6$	446.19319	5.756	11237132.47	72
(2E)-3-(4-Hydroxyphenyl)-N-[2-(4-hydroxyphenyl)ethyl] acrylamide	$C_{17}H_{17}NO_3$	283.12612	8.64	11072830.81	97.6
acridine-9(10H)-thione	$C_{13}H_9NS$	211.04604	16.919	11003620.19	96.2
α-Linolenic acid	$C_{18}H_{30}O_2$	278.22984	19.864	10775702.87	90.8
N-{4-[(2R,3R)-3-(Hydroxymethyl)-4-methyl-5-oxo-2-morpholinyl]phenyl}cyclopentanecarboxamide	$C_{18}H_{24}N_2O_4$	310.18625	13.432	9945118.90	73.6
NP-018660	$C_{13}H_{22}O_3$	208.14699	4.713	9400460.00	70.3
2-{5-[2-({2-[5-(2-hydroxybutyl)oxolan-2-yl]propanoyl}oxy) butyl]oxolan-2-yl}propanoic acid	$C_{22}H_{38}O_7$	436.24498	5.643	9090370.31	79.9
3,5-di-tert-Butyl-4-hydroxybenzoic acid	$C_{15}H_{22}O_3$	250.16001	14.647	9058908.30	89.1
(2R,3R,3aR,5R)-5,7-dimethoxy-2-(7-methoxy-2H-1,3-benzodioxol-5-yl)-3-methyl-3a-(prop-2-en-1-yl)-2,3,3a,4,5,6-hexahydro-1-benzofuran-6-one	$C_{22}H_{26}O_7$	424.14913	5.107	8868358.48	93.3
Testosterone enanthate	$C_{26}H_{40}O_3$	400.29381	16.612	8428883.59	82.6
Cafestol	$C_{20}H_{28}O_3$	294.22748	17.376	8303757.42	90.1
Galaxolidone	$C_{18}H_{24}O_2$	272.18181	17.739	7844401.72	97.7

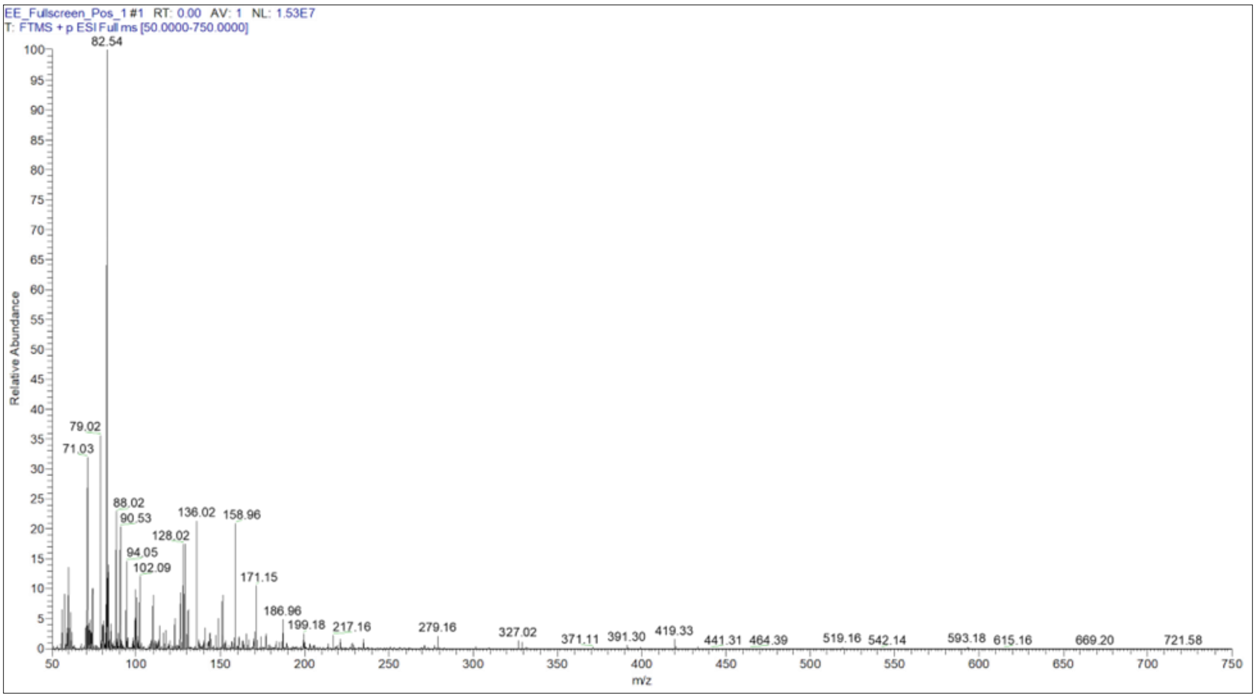


Fig. 7. LC-HRMS chromatogram of chemical components leaves of *Dracaena trifasciata* isolate potential fraction.

Table 6. Results of Identification of active compound from potential fraction isolate of *Dracaena trifasciata* leaves using LC-HRMS

Name	Formula	Molecular weight	RT [min]	Area (Max.)	mzCloud best match
Hexamethylenetetramine	$C_6H_{12}N_4$	140.10607	26.399	313014204.26	98
Dibutyl phthalate	$C_{16}H_{22}O_4$	278.15797	17.815	196840564.89	99.5
2-[(3S)-1-(Cyclohexylmethyl)-3-pyrrolidinyl]-1H-benzimidazole-5-carbonitrile	$C_{19}H_{24}N_4$	308.20305	18.804	164560800.04	92.6
1,2,3,4-tetrahydronaphthalen-1-one 1-(4,5-dihydro-1H-imidazol-2-yl)hydrazone	$C_{13}H_{16}N_4$	228.13599	7.748	160322352.85	74.8
Diisobutylphthalate	$C_{16}H_{22}O_4$	278.158	17.701	86292875.81	99.4
Palmitic Acid	$C_{16}H_{32}O_2$	273.27101	14.272	76918098.37	83.7
NP-019811	$C_6H_7NO_2$	125.04794	0.899	70022600.09	95,1
4-Methoxycinnamic acid	$C_{10}H_{10}O_3$	178.06304	20.503	35495174.80	98.3
Triethylene glycol monobutyl ether	$C_{10}H_{22}O_4$	206.15253	7.748	32597157.49	98.5
6-Ketoprostaglandin F1α	$C_{20}H_{34}O_6$	370.23263	0.892	30209520.83	98
Nicotinamide	$C_6H_6N_2O$	122.04823	0.897	30007576.76	90.3
N-[3-(tert-butyl)-1-methyl-1H-pyrazol-5-yl]-N'-[4-(trifluoromethoxy)phenyl]urea	$C_{16}H_{19}F_3N_4O_2$	356.14989	14.269	29138542.30	97
Galaxolidone	$C_{18}H_{24}O_2$	272.18181	17.726	23329991.31	97.6
N4-phenethylmorpholine-4-carbothioamide	$C_{13}H_{18}N_2OS$	272.09169	0.888	21037664.30	89.6
N2-(2-Benzoylphenyl)-1-benzylpyrrolidine-2-carboxamide	$C_{25}H_{24}N_2O_2$	384.18328	15.844	18975999.56	72.9
5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine	$C_{20}H_{14}N_4$	310.1259	13.658	16918418.09	82.8
acridine-9(10H)-thione	$C_{13}H_9NS$	211.04617	16.917	16754677.25	97.1
3-Hydroxy-2-methylpyridine	C_6H_7NO	109.05309	0.912	15415294.59	72.8
NP-008882	$C_{27}H_{48}O_9$	538.30443	21.481	15326460.12	60.9
(2E)-3-(4-Hydroxyphenyl)-N-[2-(4-hydroxyphenyl)ethyl] acrylamide	$C_{17}H_{17}NO_3$	283.12633	8.459	14722729.05	97.9
Reserpine	$C_{33}H_{40}N_2O_9$	608.28459	21.004	13971588.13	76.2
Adenosine	$C_{10}H_{13}N_5O_4$	267.10124	0.88	13513216.32	99.6
Caprolactam	$C_6H_{11}NO$	113.08432	0.901	13216251.50	91.5
N-Butylbenzenesulfonamide	$C_{10}H_{15}NO_2S$	213.08302	4.842	12241048.96	87.4
Triethylene glycol monobutyl ether	$C_{10}H_{22}O_4$	206.15253	7.653	10892892.91	93.9
Despropionyl p-fluoro fentanyl	$C_{19}H_{23}FN_2$	298.18236	12.493	8216037.12	66.2
(2R,3S,4S,5S,6R)-2-({7-hydroxy-4-[(3Z)-5-hydroxy-3-methylpent-3-en-1-yl]-4a,8,8-trimethyl-3-methylidene-decahydronaphthalen-2-yl}oxy)-6-(hydroxymethyl) oxane-3,4,5-triol	$C_{26}H_{44}O_8$	484.30469	19.421	7766157.33	92.4
5-(6-hydroxy-6-methyloctyl)-2,5-dihydrofuran-2-one	$C_{13}H_{22}O_3$	208.1471	0.887	7512596.44	68.2
Ostruthin	$C_{19}H_{22}O_3$	298.16112	16.871	7482827.91	65.7
N-{4-[(2R,3R)-3-(Hydroxymethyl)-4-methyl-5-oxo-2-morpholinyl]phenyl}cyclopentanecarboxamide	$C_{18}H_{24}N_2O_4$	310.1865	13.417	6886312.13	73.7
1-[3-Methyl-5-(2-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl]ethan-1-one	$C_{18}H_{15}N_3O_3$	321.10607	6.853	6881169.67	87.2
Betaine	$C_5H_{11}NO_2$	117.07919	0.849	5981245.62	89.2

only substantiates *D. trifasciata* leafs' antibacterial potential but also provides new information through an identification of certain active fractions and supporting evidence of the mechanism at the cell and molecular levels. Further, LC-HRMS, as an analytical technique to identify molecules and cell/molecular assays (such as SEM observation, inhibition of biofilm and gene expression study), provides mechanism-driven information not furnished in prior studies.

The subsequent TLC-preparative separation of fraction 3, leading to isolation of 4 isolates, also showed promise for these compounds. The most active compound was fraction 1, showing that specific isolation can raise both purity and strength of antimicrobial agents. The raised strength in separated fractions suggests certain compounds may have principal roles in bacterial cell lysis, presumably through cell wall and cell membrane integrity disruption (15). Ultimately, these findings highlight that fraction 3 of *D. trifasciata* leaves contains potent antibacterial compounds, which were further concentrated in potential fraction 1, making it a promising candidate for chemical characterisation and future drug development. SEM observation of *S. aureus* cell morphology showed cell destruction in bacterial cells caused by treatment with potential fractions. The cell destruction indicates and confirms cell structure damaging potential of cell structure damaging cell structure damaging cell structure damaging bioactive compounds in *D. trifasciata*. Thus, it can be accounted for in terms of cell wall disruption based on antibacterial activity, increased membrane permeability resulting in cell content loss, inactivation of cell metabolism enzymes and even functional gene damage. (34). This process can occur due to the attachment of antimicrobial compounds to the surface of microbial cells or diffusion into the cells (44).

The presence of antibacterial activity suggests the existence of bioactive compounds in the extract of *D. trifasciata*. This activity is possibly attributed to the presence of alkaloids, saponins, terpenoids, steroids, glycosides, tannins, organic acids, fats and oils (36,37). LC-MS/MS analysis of *D. trifasciata* leaf ethanolic extract subfractions obtained 7 compounds, including alkaloids - 1-acetyl-b-carboline, methyl pyrophaeophorbide A, oliveramine, flavonoids - (2S)-30, 40- methylenedioxy-5, 7-dimethoxyflavane, monoterpenes digiprolactone, phenolic methyl gallate and trichosanic acid. Furthermore, 5-methyl-11-(2-oxopyridin-1(2H)-yl)undecaneperoxoic acid was also isolated from the extract (38). GC-MS analysis showed various phytochemical constituents, namely 17-pentatriacontene, corynan-17-ol, 18,19-didehydro-10- methoxy-, acetate (ester), 3-(octadecyloxy) propyl ester, 7-methyl-ztetradecen-1-ol acetate, oleic acid, cis-13-eicosenoic acid, citronellol, tert-hexadecanethio, 1-monolinoleoylglycerol trimethylsilyl ether, ethyl iso-allocholate, 1-heptatriacotanol, E, E, Z1,3,12-nonadecatriene-5,14-diol, 2,3-bis[(trimethylsilyl)oxy] propyl ester, (Z, Z, Z), rhodopin, 3-pyridinecarboxylic acid and 14,15:21,23- diepoxy-7- hydroxy-4,4,8-trimethyl-, (5`a,7`a,13`a,14`a,15`a,17aa`)-(45).

The identification of key bioactive compounds, including 4-coumaric acid, catechin and α-linolenic acid, among others, establishes a foundation for future study. The consistent presence of these compounds across extracts, fractions and potential isolates signifies the potential role in the antibacterial properties observed. Understanding their specific contributions to antibacterial activity can inform the development of novel antimicrobial therapies. Several identified compounds, such as hexamethylenetetramine, reserpine and palmitic acid, are important bioactive substances in antibacterial activity (5, 46, 47).

Hexamethylenetetramine is a versatile compound with significant applications in medicine and industry. Its antimicrobial properties have proven useful in the treatment of urinary tract infections, primarily due to the ability to release formaldehyde in acidic urine. This release produces an antiseptic effect within the urinary tract, reducing bacterial populations. In clinical settings, hexamethylenetetramine may also be administered alongside other medications to alleviate pain and spasms associated with UTIs. Additionally, the compound plays a critical role in resin production and various industrial processes, affirming its multifaceted value (48, 49).

Reserpine, an alkaloid commonly referred to as Indian snakeroot, holds a long-standing place in traditional medicine and remains relevant in modern pharmacology. It has been adopted in the management of hypertension and certain psychiatric conditions by diminishing sympathetic nervous system activity, which lowers heart rate and promotes vasodilation. In addition to these therapeutic effects, reserpine has antimicrobial, antivirulence and antibiofilm activities against *S. aureus* bacteria. Evidence suggests that the effects are mediated through interference with quorum-sensing regulatory proteins, biofilm-associated proteins and virulence gene regulators (24, 50).

Palmitic acid is among the most readily obtainable fatty acids from plants and functions as a precursor in fatty acid biosynthesis. The majority of compounds detected in the extracts, fractions and potential isolates belong to the fatty acid group, which is recognised for its role in antimicrobial activity in plants (51). Palmitic acid contributes to inhibiting bacterial growth through a bactericidal mechanism, primarily by damaging bacterial cell membranes (52). In addition to the antimicrobial function, it also possesses anticancer and antioxidant properties (51). Several studies showed that α-eleostearic acid and α-linolenic acid had antifungal and antibacterial properties (53, 54).

The results of this study indicate a progressive decrease in antibacterial activity from the extract to the fractions and isolates. This simplification of compound profiles was confirmed in LC-HRMS analysis. Simplification occurs due to separating the extract into fractions to reduce the complexity of the compounds in the extract. The possibility of synergy can be investigated through this fractionation. The reduced activity with fractionation allows for the measurement of synergy, which will support further studies. Further studies on the isolation and characterisation of active compounds with antibacterial activity and even potential applications in the healthcare sector, are also possible.

Further research is needed to confirm synergistic effects on compounds suspected of playing a role in antibacterial activity, as decreased activity during fractionation suggests potential interactions. Furthermore, exploring the mechanisms of antibacterial action will provide supporting knowledge. Formulation and stability assay of active compounds, as well as in vivo efficacy and toxicity assessments, are fundamental before their application as potential therapeutic agents. Thus, this research advances the field by moving beyond descriptive phytochemistry towards targeted isolation and characterisation of antibacterial lead compounds from *D. trifasciata*, laying the groundwork for future development of plant-derived antimicrobial agents.

Conclusion

This study demonstrated that the leaf extract of *D. trifasciata* exhibits significant antibacterial activity against *S.aureus*, as evidenced by inhibition zone assays and SEM observations of bacterial cell damage. Bioassay-guided fractionation effectively traced the antibacterial activity from crude extracts to isolated fractions, leading to the identification of several active compounds, including hexamethylenetetramine, reserpine, palmitic acid and adenosine. These findings affirm the importance of fractionation in discovering effective antibacterial agents from plant sources. However, the present findings are limited to *in vitro* assays and the biological activities of the identified compounds were not individually validated. Moreover, potential cytotoxicity and *in vivo* efficacy remain to be elucidated. Future research should therefore focus on detailed chemical characterisation of the most potent fractions, isolation of the bioactive constituents and investigation of their mechanisms of action in both *in vitro* and *in vivo* models. Collectively, this study provides a scientific basis for the development of new plant-derived antimicrobial agents to address the growing challenge of antibiotic resistance.

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Authors' contributions

WFD conceived and coordinated the research, provided materials and tools, carried out statistical analysis and discussion on antibacterials and reviewed compound identification with LC-HRMS. NT performed fractionation studies, analysed and discussed antibacterial and LC-HRMS test results, and provided advice on the research and writing process. NAL contributed to antibacterial testing and the fractionation process. SDK contributed to the data analysis process and wrote the discussion. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare

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