RESEARCH PAPER

Antibacterial, Bactericidal and Antibiofilm of 1-Hexadecanoyl-sn-glycerol Against Multidrug Resistant Clinical Isolates

Ganta Prashanthi[1*]

[1]Department of Botany, P.V.K.N. Government College (Autonomous), Chittoor - 517002, Andhra Pradesh, India

***Corresponding author: Ganta Prashanthi, Department of Botany, P.V.K.N. Government College (Autonomous), Chittoor - 517002, Andhra Pradesh, India**

Email: Prashanthi.gps@gmail.com

Received: 30th May, 2026; **Revised:** 8th June, 2026; **Accepted:** 10th June, 2026; **Available Online:** 12th June, 2026

ABSTRACT

The emergence of multidrug-resistant (MDR) bacterial pathogens has become a major global health concern, necessitating the exploration of novel antimicrobial agents from natural sources. In the present study, 1-Hexadecanoyl-sn-glycerol (1-monopalmitin) was isolated from the methanolic leaf extract of Dendrophthoe falcata through bioassay-guided fractionation. The crude methanolic extract was subjected to silica gel column chromatography, and active fractions were purified using thin-layer chromatography (TLC). The purified compound identified as 1-Hexadecanoyl-sn-glycerol. The antibacterial activity of the isolated compound was evaluated against clinically important multidrug-resistant pathogens, including methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Staphylococcus aureus (VRSA), Pseudomonas aeruginosa, and Klebsiella pneumoniae. The compound demonstrated concentration-dependent antibacterial activity, with inhibition zones ranging from 6.5 ± 0.2 mm to 20.5 ± 0.9 mm. Minimum inhibitory concentration (MIC) values ranged between 62.5 and 125 µg/mL, while minimum bactericidal concentration (MBC) values ranged from 125 to 250 µg/mL. The isolated compound exhibited greater activity against Gram-positive bacteria, particularly MRSA and VRSA. Furthermore, 1-Hexadecanoyl-sn-glycerol significantly inhibited biofilm formation, achieving biofilm inhibition rates of up to 82.7% against MRSA. Membrane integrity studies revealed substantial leakage of intracellular nucleic acids and proteins, indicating membrane disruption as the primary antibacterial mechanism.

Keywords: Dendrophthoe falcata, 1-Hexadecanoyl-sn-glycerol, 1-Monopalmitin, Antibacterial activity, Multidrug-resistant bacteria, MRSA, VRSA, Biofilm inhibition, Membrane integrity, Natural antimicrobial agents, Phytochemicals, Bioassay-guided fractionation.

How to cite this article: Prashanthi G. Antibacterial, Bactericidal and Antibiofilm of 1-Hexadecanoyl-sn-glycerol Against Multidrug Resistant Clinical Isolates. Int J Drug Deliv Technol. 2026;16(58s): 1346-1355. DOI: 10.25258/ijddt.16.58s.143

Source of support: Nil

Conflict of interest: None

1.0 Introduction

The rapid emergence and spread of antimicrobial resistance (AMR) have become one of the most serious threats to global public health. The indiscriminate use of antibiotics in human medicine, veterinary practice, and agriculture has accelerated the development of multidrug-resistant (MDR) pathogens, thereby reducing the effectiveness of conventional antimicrobial therapies. According to the World Health Organization (WHO, 2023), antimicrobial resistance is responsible for millions of infections annually and is projected to become one of the leading causes of mortality worldwide if effective interventions are not developed.

Among the clinically important resistant pathogens, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSA), *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* represent major healthcare challenges due to their ability to resist multiple antibiotics and form persistent biofilms (Ventola, 2015). Biofilm formation further complicates treatment because microbial cells embedded within biofilms exhibit significantly greater resistance to antimicrobial agents than their planktonic counterparts (Costerton et al., 1999).

The decline in the discovery of novel antibiotics has prompted researchers to explore alternative antimicrobial agents, including naturally occurring lipids and their derivatives. Lipid-based molecules possess unique physicochemical properties that enable them to interact directly with microbial membranes, causing structural disruption and loss of cellular integrity (Desbois and Smith, 2010). Unlike many conventional antibiotics that target specific metabolic pathways, lipid-derived compounds often exert broad-spectrum antimicrobial activity through membrane destabilization, reducing the likelihood of resistance development.

Monoglycerides, a class of glycerol esters of fatty acids, have attracted considerable attention because of their antimicrobial, antibiofilm, and membrane-active

properties. Several studies have demonstrated that monoglycerides can inhibit the growth of Gram-positive and Gram-negative bacteria by integrating into lipid bilayers and altering membrane permeability (Kabara et al., 1972; Bergsson et al., 2001). Among these compounds, 1-hexadecanoyl-sn-glycerol (1-monopalmitin), a monoacylglycerol derived from palmitic acid, possesses an amphiphilic structure consisting of a hydrophobic fatty acid chain and a hydrophilic glycerol moiety. This structural arrangement may facilitate interactions with bacterial cell membranes, resulting in disruption of membrane integrity and inhibition of microbial growth.

In addition to antibacterial activity, lipid-based compounds have shown promise in controlling biofilm formation. Biofilms are complex microbial communities embedded within an extracellular polymeric matrix that protects bacterial cells from environmental stress and antimicrobial agents (Hall-Stoodley et al., 2004). The ability of amphiphilic lipids to penetrate and destabilize biofilm structures suggests their potential application as antibiofilm agents against MDR pathogens.

Despite extensive investigations on various monoglycerides, the biological potential of 1-hexadecanoyl-sn-glycerol remains insufficiently explored, particularly against multidrug-resistant clinical isolates. Furthermore, limited information is available regarding its effects on bacterial biofilms, membrane integrity, and microbial killing kinetics. Therefore, a comprehensive investigation of the antibacterial, antibiofilm, and time-kill dynamics of 1-hexadecanoyl-sn-glycerol is warranted. The continuous search in extraction and characterization of phytochemicals form different medicinal plants and their pharmacological potential determination especially with antibacterial or anticancer holds great importance now- a- days (Thupurani et al., 2013; Murali Krishna Thupurani et al., 2013_a,; Murali Krishna Thupurani et al., 2013_b; Gorripati et al., 2018; Thupurani Murali Krishna, 2018; Racha Srikanth et al., 2020; Gujjari Sreehitha Pratap et al., 2021; Challa Surekha et al., 2022; Srilekha Konakanchi et al., 2023; Praveen Kumar, Thupurani Murali Krishna, 2024; Ganta Prashanthi, 2024; Ganta Prashanthi, 2025).

The present study aims to evaluate the antibacterial efficacy of 1-hexadecanoyl-sn-glycerol against clinically relevant multidrug-resistant pathogens, assess its ability to inhibit biofilm formation, and investigate its potential mechanism of action through membrane-targeting effects. The findings of this study may contribute to the development of lipid-based antimicrobial agents as alternative therapeutic strategies for combating antimicrobial resistance.

2.0 Materials and Methods

2.1 Chemicals and Reagents

1-Hexadecanoyl-sn-glycerol (1-monopalmitin) was used as the test compound in the present study. Mueller-Hinton agar (MHA), Mueller-Hinton broth (MHB), crystal violet, phosphate-buffered saline (PBS), dimethyl sulfoxide (DMSO), and all analytical-grade chemicals were procured from standard commercial suppliers. Gentamicin was employed as the reference antibiotic in antibacterial assays.

2.2 Extraction, Isolation and Structural Characterization of 1-Hexadecanoyl-sn-glycerol

Fresh leaves of *Dendrophthoe falcata* were collected, authenticated, washed thoroughly with distilled water, shade-dried at room temperature, and pulverized into a fine powder using a mechanical grinder. The powdered plant material was subjected to extraction with methanol by Soxhlet apparatus for 72 h (Malipeddi Supriya and Thupurani Murali Krishna, 2024_a). The extract was filtered through Whatman No. 1 filter paper, and the solvent was removed under reduced pressure using a rotary evaporator to obtain the crude methanolic extract. The crude methanolic extract was initially screened for antibacterial activity against selected clinical pathogens. Based on the bioactivity results, the methanolic extract was selected for bioassay-guided fractionation (Malipeddi supriya and Thupurani Murali Krishna, 2024_b). The extract was subjected to silica gel column chromatography packed with silica gel (60–120 mesh) as the stationary phase. Elution was performed using solvent systems of increasing polarity consisting of hexane, hexane:ethyl acetate mixtures, ethyl acetate, and methanol. Fractions were collected sequentially and monitored for antibacterial activity. Fractions exhibiting negligible or no biological activity were excluded from further investigation, while active fractions were pooled for purification.

The active pooled fractions were analyzed by thin-layer chromatography (TLC) using silica gel 60 F254 precoated aluminum plates. Appropriate solvent systems were employed to achieve optimal separation. The developed chromatograms were visualized under ultraviolet light (254 and 366 nm) and further examined after spraying with suitable detecting reagents. Fractions exhibiting similar chromatographic profiles were combined. Repeated chromatographic purification yielded a compound displaying a single distinct spot on TLC, indicating chromatographic purity.

The purified compound was subjected to spectroscopic characterization for structural elucidation. Proton nuclear magnetic resonance (^1H NMR) and carbon-13 nuclear magnetic resonance (^13C NMR) spectra were recorded using deuterated solvents and tetramethylsilane (TMS) as an internal standard. The spectral data obtained from ^1H NMR

and ^13C NMR analyses were interpreted and compared with published literature data. Based on the characteristic chemical shifts, multiplicity patterns, carbon signals, and spectral correlations, the isolated compound was identified as **1-Hexadecanoyl-sn-glycerol (1-monopalmitin)**. The purity of the isolated compound was confirmed by the presence of a single spot on TLC and well-resolved NMR spectra without detectable impurities. The isolated compound was subsequently used for antibacterial, antibiofilm, and mechanistic investigations.

2.3 Microbial Strains and Culture Conditions

The antibacterial activity of 1-hexadecanoyl-sn-glycerol was evaluated against multidrug-resistant clinical isolates including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSA), *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Bacterial cultures were maintained on Mueller-Hinton agar slants at 4°C and subcultured periodically. Prior to each experiment, fresh bacterial cultures were grown overnight in Mueller-Hinton broth at 37°C. The inoculum density was adjusted to 0.5 McFarland standard, corresponding to approximately 1×10^8 CFU/mL, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2020).

2.4 Antibacterial Activity Assay

The antibacterial activity of 1-hexadecanoyl-sn-glycerol was determined using the agar well diffusion method described by Bauer et al. (1966). Briefly, sterile Mueller-Hinton agar plates were uniformly inoculated with bacterial suspensions using sterile cotton swabs. Wells of 6 mm diameter were aseptically punched into the agar and filled with different concentrations of the test compound. Gentamicin and DMSO served as positive and negative controls, respectively. The plates were incubated at 37°C for 24 h, after which the diameters of the inhibition zones were measured and recorded in millimeters.

2.5 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of 1-hexadecanoyl-sn-glycerol was determined by the broth microdilution method according to CLSI guidelines (CLSI, 2020). Serial two-fold dilutions of the compound were prepared in sterile 96-well microtiter plates containing Mueller-Hinton broth. Each well was inoculated with standardized bacterial suspension and incubated at 37°C for 24 h. The MIC was defined as the lowest concentration of the compound that completely inhibited visible bacterial growth.

2.6 Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was determined from the MIC assay. Aliquots from wells exhibiting no visible growth were streaked onto Mueller-Hinton agar plates and incubated at 37°C for 24 h. The lowest concentration showing complete absence of bacterial colonies was recorded as the MBC according to CLSI recommendations (CLSI, 2020).

2.7 Biofilm Inhibition Assay

The antibiofilm activity of 1-hexadecanoyl-sn-glycerol was evaluated using the crystal violet microtiter plate method described by Stepanović et al. (2000). Briefly, bacterial cultures were inoculated into sterile 96-well polystyrene microplates containing tryptic soy broth supplemented with glucose and different concentrations of the test compound. Following incubation at 37°C for 24 h, the planktonic cells were removed and the wells were gently washed three times with sterile phosphate-buffered saline. The adhered biofilms were stained with 0.1% crystal violet solution for 15 min. Excess stain was removed by washing with distilled water, and the retained dye was solubilized using ethanol. Biofilm biomass was quantified by measuring absorbance at 595 nm using a microplate reader.

2.8 Membrane Integrity Assay

The effect of 1-hexadecanoyl-sn-glycerol on bacterial membrane integrity was investigated by measuring the leakage of intracellular components as described by Desbois and Smith (2010). Bacterial cells treated with different concentrations of the compound were incubated for a specified period and subsequently centrifuged. The absorbance of the resulting supernatants was measured at 260 nm and 280 nm to quantify the release of nucleic acids and proteins, respectively. Increased absorbance values were interpreted as evidence of membrane disruption and cellular leakage.

2.9 Statistical Analysis

All experiments were performed in triplicate ($n = 3$), and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Differences were considered statistically significant at $p < 0.05$.

3.0 Result

3.1 Antibacterial Activity of 1-Hexadecanoyl-sn-glycerol Against Multidrug-Resistant Clinical Isolates

The antibacterial potential of the purified compound, **1-Hexadecanoyl-sn-glycerol**, isolated from the methanolic leaf extract of *Dendrophthoe falcata*, was evaluated against multidrug-resistant clinical isolates including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus*

aureus (VRSA), *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* using the agar well diffusion method. The compound exhibited a concentration-dependent inhibitory effect against all tested bacterial pathogens, with the diameter of the inhibition zones increasing progressively with increasing concentrations of the compound (Table 1).

At the lowest tested concentration (25 µg/well), the compound produced inhibition zones ranging from 6.5 ± 0.2 mm to 8.2 ± 0.4 mm. A gradual enhancement in antibacterial activity was observed as the concentration increased, reaching maximum inhibition zones of 20.5 ± 0.9 mm against MRSA, 19.7 ± 0.8 mm against VRSA, 16.3 ± 0.7 mm against *Pseudomonas aeruginosa*, and 16.8 ± 0.7 mm against *Klebsiella pneumoniae* at 250 µg/well. Among the tested pathogens, MRSA exhibited the highest susceptibility to 1-Hexadecanoyl-sn-glycerol, followed by VRSA, whereas *P. aeruginosa* demonstrated the lowest susceptibility throughout the concentration range tested.

The antibacterial efficacy of the compound was particularly pronounced against Gram-positive bacteria, as evidenced by the larger inhibition zones observed against MRSA and VRSA compared with Gram-negative organisms. This differential susceptibility may be attributed to structural variations in the bacterial cell envelope. Gram-negative bacteria possess an outer lipopolysaccharide membrane that serves as an additional permeability barrier, thereby restricting the penetration of hydrophobic antimicrobial compounds. In contrast, Gram-positive bacteria lack this outer membrane, facilitating greater interaction of lipid-based molecules with the cytoplasmic membrane (Desbois and Smith, 2010).

The observed antibacterial activity of 1-Hexadecanoyl-sn-glycerol may be associated with its amphiphilic molecular architecture comprising a hydrophobic palmitoyl chain and a hydrophilic glycerol moiety. Such structural characteristics are known to promote insertion into bacterial lipid bilayers, leading to membrane destabilization, increased permeability, leakage of intracellular constituents, and ultimately bacterial cell death. Similar antimicrobial mechanisms have been reported for fatty acids and monoglycerides by Kabara et al. (1972) and Bergsson et al. (2001), who demonstrated that lipid-derived compounds exert bactericidal effects primarily through disruption of membrane integrity.

The antibacterial performance exhibited by 1-Hexadecanoyl-sn-glycerol against multidrug-resistant clinical isolates highlights its potential as a promising lipid-based antimicrobial agent. The significant inhibitory activity observed against MRSA and VRSA is particularly noteworthy, considering the increasing prevalence of antibiotic-resistant staphylococcal infections and the urgent need for alternative therapeutic strategies.

Table 1. Antibacterial activity of 1-Hexadecanoyl-sn-glycerol against multidrug-resistant clinical isolates

Concentration (µg/well)	MRSA (mm)	VRSA (mm)	*P. aeruginosa* (mm)	*K. pneumoniae* (mm)
DMSO (Negative Control)	ND	ND	ND	ND
25	8.2 ± 0.4	7.8 ± 0.3	6.5 ± 0.2	6.8 ± 0.3
50	10.4 ± 0.5	9.8 ± 0.4	8.1 ± 0.4	8.5 ± 0.4
100	13.6 ± 0.6	12.9 ± 0.5	10.2 ± 0.5	10.8 ± 0.5
150	15.8 ± 0.7	15.1 ± 0.6	12.4 ± 0.4	12.9 ± 0.5
200	18.3 ± 0.8	17.6 ± 0.7	14.8 ± 0.6	15.2 ± 0.6
250	20.5 ± 0.9	19.7 ± 0.8	16.3 ± 0.7	16.8 ± 0.7
Gentamicin (10 µg/disc)	24.8 ± 0.5	23.6 ± 0.6	22.4 ± 0.4	23.1 ± 0.5
Values are expressed as mean ± SD (n = 3). ND = No detectable inhibition zone.				

3.2 Minimum Inhibitory Concentration

he minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 1-Hexadecanoyl-sn-glycerol against multidrug-resistant clinical isolates are presented in Table 2. The compound demonstrated appreciable antibacterial potency against all tested pathogens, with MIC values ranging from 62.5 to 125 µg/mL. The lowest MIC value (62.5 µg/mL) was observed against MRSA and VRSA, indicating greater susceptibility of Gram-positive bacteria to the isolated compound. In contrast, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

exhibited MIC values of 125 µg/mL, suggesting relatively lower sensitivity.

Similarly, the MBC values ranged between 125 and 250 µg/mL. Complete bactericidal activity against MRSA and VRSA was achieved at 125 µg/mL, whereas 250 µg/mL was required to eliminate *P. aeruginosa* and *K. pneumoniae*. The MBC/MIC ratio of approximately 2 for all tested organisms indicates that 1-Hexadecanoyl-sn-glycerol exhibits a bactericidal mode of action rather than merely inhibiting bacterial growth.

The enhanced susceptibility of Gram-positive pathogens may be attributed to the direct interaction of the amphiphilic monoglyceride with the bacterial cytoplasmic membrane, leading to membrane destabilization and leakage of intracellular components. These findings are consistent with previous reports by Kabara et al. (1972), Bergsson et al. (2001), and Desbois and Smith (2010), who demonstrated that fatty acids and monoglycerides exert antimicrobial effects primarily through membrane disruption.

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of 1-Hexadecanoyl-sn-glycerol against multidrug-resistant clinical isolates

Test Organism	MIC (µg/mL)	MBC (µg/mL)
MRSA	62.5	125
VRSA	62.5	125
Pseudomonas aeruginosa	125	250
Klebsiella pneumoniae	125	250
Gentamicin	1.0	2.0

Values represent the lowest concentration required to inhibit visible bacterial growth (MIC) and to completely kill the bacterial population (MBC).

3.3 Minimum Bactericidal Concentration

The minimum bactericidal concentration (MBC) of 1-Hexadecanoyl-sn-glycerol was determined to evaluate its killing efficacy against multidrug-resistant bacterial pathogens. As shown in Table 3, the compound exhibited bactericidal activity against all tested microorganisms, with MBC values ranging from 125 to 250 µg/mL. The lowest MBC value of 125 µg/mL was observed against MRSA and VRSA, indicating greater susceptibility of Gram-positive bacteria to the isolated monoglyceride. In contrast, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* required a higher concentration (250 µg/mL) for complete bacterial eradication.

The MBC values were consistently two-fold higher than the corresponding MIC values, yielding an MBC/MIC ratio of approximately 2. Such a ratio is generally indicative of a bactericidal mode of action, suggesting that 1-Hexadecanoyl-sn-glycerol is capable of killing bacterial cells rather than merely inhibiting their growth. The observed bactericidal effect may be attributed to the amphiphilic nature of the molecule, which facilitates interaction with membrane phospholipids, resulting in membrane destabilization, increased permeability, leakage of intracellular contents, and eventual cell death. Similar bactericidal mechanisms have been reported for fatty acid esters and monoglycerides by Kabara et al. (1972) and Bergsson et al. (2001).

The greater susceptibility observed in MRSA and VRSA compared to Gram-negative pathogens may be associated with the absence of an outer lipopolysaccharide membrane in Gram-positive bacteria, allowing enhanced penetration and membrane interaction of the lipid-based compound. These findings further support the potential application of 1-Hexadecanoyl-sn-glycerol as a promising antibacterial agent against clinically important multidrug-resistant pathogens.

Table 3. Minimum Bactericidal Concentration (MBC) of 1-Hexadecanoyl-sn-glycerol against multidrug-resistant clinical isolates

Test Organism	MBC (µg/mL)
MRSA	125
VRSA	125
Pseudomonas aeruginosa	250
Klebsiella pneumoniae	250
Gentamicin	2.0

3.4 Antibiofilm Activity of 1-Hexadecanoyl-sn-glycerol

The antibiofilm potential of 1-Hexadecanoyl-sn-glycerol was assessed against multidrug-resistant clinical isolates using the crystal violet microtiter plate assay. The compound demonstrated a concentration-dependent inhibition of biofilm formation in all tested bacterial pathogens (Table 4). Biofilm inhibition increased significantly with increasing concentrations of the compound, indicating its ability to interfere with bacterial adhesion and biofilm maturation processes.

At the lowest tested concentration (31.25 µg/mL), the compound exhibited biofilm inhibition ranging from 15.6 ± 1.1% in *Pseudomonas aeruginosa* to 22.4 ± 1.2% in MRSA. A marked increase in antibiofilm activity was observed at higher concentrations, reaching maximum inhibition values of 82.7 ± 2.4%, 79.8 ± 2.3%, 68.5 ± 2.2%, and 71.4 ± 2.0% against MRSA, VRSA, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, respectively, at 250 µg/mL.

Among the tested pathogens, MRSA exhibited the highest susceptibility to the antibiofilm effects of 1-Hexadecanoyl-sn-glycerol, followed closely by

VRSA. Comparatively lower inhibition was observed in Gram-negative bacteria, particularly *P. aeruginosa*, which is well known for its robust biofilm-forming capacity and complex extracellular polymeric matrix. Nevertheless, the substantial inhibition observed against all tested organisms demonstrates the broad-spectrum antibiofilm potential of the isolated compound.

The pronounced antibiofilm activity may be attributed to the amphiphilic nature of 1-Hexadecanoyl-sn-glycerol. The hydrophobic palmitoyl chain may interact with bacterial membranes and extracellular polymeric substances, while the glycerol moiety facilitates interactions with biofilm-associated components. Such interactions may disrupt initial bacterial adhesion, interfere with cell-to-cell communication, and impair biofilm maturation. Similar antibiofilm properties have been reported for lipid-derived antimicrobial compounds and monoglycerides (Bergsson et al., 2001; Desbois and Smith, 2010).

The findings suggest that 1-Hexadecanoyl-sn-glycerol possesses significant antibiofilm activity against multidrug-resistant pathogens and may serve as a promising candidate for controlling biofilm-associated infections.

Table 4. Antibiofilm activity of 1-Hexadecanoyl-sn-glycerol against multidrug-resistant clinical isolates

Concentration (µg/mL)	MRSA	VRSA	*P. aeruginosa*	*K. pneumoniae*
31.25	22.4 ± 1.2	20.8 ± 1.4	15.6 ± 1.1	17.2 ± 1.3
62.5	41.8 ± 1.8	39.6 ± 1.7	31.4 ± 1.5	33.7 ± 1.6
125	63.5 ± 2.1	60.9 ± 2.0	49.3 ± 1.9	52.1 ± 1.8
250	82.7 ± 2.4	79.8 ± 2.3	68.5 ± 2.2	71.4 ± 2.0
Values are expressed as mean ± SD (n = 3). Mean=Percentage Biofilm Inhibition (%)				

3.5 Effect of 1-Hexadecanoyl-sn-glycerol on Bacterial Membrane Integrity

To investigate the possible mechanism underlying the antibacterial activity of 1-Hexadecanoyl-sn-glycerol, membrane integrity assays were performed by measuring the leakage of intracellular nucleic acids and proteins from treated bacterial cells. Membrane damage was assessed by monitoring the absorbance of the extracellular medium at 260 nm and 280 nm, corresponding to the release of nucleic acids and proteins, respectively. The results demonstrated a concentration-dependent increase in the leakage of intracellular components in all tested bacterial pathogens, indicating significant disruption of membrane integrity following treatment with the isolated compound (Table 5).

Among the tested organisms, MRSA and VRSA exhibited the highest levels of membrane damage, as evidenced by increased absorbance values at both wavelengths. At the highest tested concentration (250 µg/mL), the absorbance at 260 nm increased from 0.112 ± 0.008 to 0.684 ± 0.021 in MRSA and from 0.105 ± 0.007 to 0.651 ± 0.019 in VRSA. Similarly, protein leakage measured at 280 nm increased substantially in treated cells compared to untreated controls. Gram-negative bacteria also exhibited significant membrane disruption, although the magnitude of leakage was comparatively lower than that observed in Gram-positive pathogens.

The observed increase in extracellular nucleic acid and protein content suggests that 1-Hexadecanoyl-sn-glycerol compromises bacterial membrane permeability, resulting in the release of intracellular constituents. These findings support the hypothesis that the antibacterial activity of the compound is mediated through membrane destabilization. The amphiphilic structure of 1-Hexadecanoyl-sn-glycerol, comprising a hydrophobic palmitoyl chain and a hydrophilic glycerol moiety, may facilitate insertion into the lipid bilayer, causing membrane disorganization and eventual cell lysis. Similar membrane-targeting mechanisms have been reported for monoglycerides and fatty acid derivatives by Kabara et al. (1972), Bergsson et al. (2001), and Desbois and Smith (2010).

The membrane integrity assay findings are in agreement with the antibacterial, MIC, MBC, and antibiofilm results, further confirming that membrane disruption represents a major mechanism by which 1-Hexadecanoyl-sn-glycerol exerts its antimicrobial effects against multidrug-resistant bacterial pathogens.

Table 5. Effect of 1-Hexadecanoyl-sn-glycerol on membrane integrity of multidrug-resistant bacterial pathogens

Organism	Conc. (µg/mL)	A260 nm (Nucleic Acid Leakage)	A280 nm (Protein Leakage)
MRSA	Control	0.112 ± 0.008	0.086 ± 0.005
	62.5	0.284 ± 0.012	0.215 ± 0.010
	125	0.472 ± 0.018	0.356 ± 0.014

	250	0.684 ± 0.021	0.521 ± 0.018
VRSA	Control	0.105 ± 0.007	0.081 ± 0.004
	62.5	0.261 ± 0.011	0.198 ± 0.009
	125	0.445 ± 0.017	0.334 ± 0.013
	250	0.651 ± 0.019	0.496 ± 0.017
P. aeruginosa	Control	0.098 ± 0.006	0.073 ± 0.004
	125	0.224 ± 0.010	0.176 ± 0.008
	250	0.401 ± 0.015	0.302 ± 0.012
	500	0.592 ± 0.018	0.445 ± 0.015
K. pneumoniae	Control	0.101 ± 0.007	0.075 ± 0.004
	125	0.236 ± 0.011	0.181 ± 0.009
	250	0.418 ± 0.016	0.314 ± 0.013
	500	0.611 ± 0.019	0.458 ± 0.016

4.0 Discussion

The drug discovery against bacterial infections still remain unexplored. Despite of various drugs are available in the treatment of bacterial infections, majorly, are gaining resistant and make difficulty during therapy. Plant derived phytochemicals or novel synthetic compounds are reported to target the bacterial receptors and play important role in inhibition or suppression or bactericidal potentials (Kumaraswamy gullapelli et al., 2014; Budarapu Neelamma et al., 2016; Ramya Sucharitha et al., 2016; Amarnath Velidandi et al., 2022; Nagavelli Ramu et al., 2023; Nagavelli Ramu et al., 2024).

The increasing prevalence of multidrug-resistant (MDR) bacterial pathogens has created an urgent need for alternative antimicrobial agents capable of overcoming conventional antibiotic resistance mechanisms. In the present study, **1-Hexadecanoyl-sn-glycerol (1-monopalmitin)** isolated from the methanolic leaf extract of *Dendrophthoe falcata* demonstrated significant antibacterial, antibiofilm, and membrane-disrupting activities against clinically important MDR pathogens, including MRSA, VRSA, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The findings suggest that this naturally occurring monoglyceride may serve as a promising antimicrobial lead compound.

The antibacterial assay revealed a concentration-dependent increase in inhibitory activity against all tested pathogens. Among the bacterial isolates, MRSA and VRSA exhibited greater susceptibility than *P. aeruginosa* and *K. pneumoniae*. This observation is consistent with previous reports indicating that fatty acid esters and monoglycerides generally display stronger activity against Gram-positive bacteria than Gram-negative organisms (Kabara et al., 1972). The reduced susceptibility of Gram-negative bacteria may be attributed to the presence of an outer lipopolysaccharide membrane, which acts as an effective permeability barrier against hydrophobic antimicrobial molecules (Nikaido, 2003).

The antibacterial activity observed in the present investigation may be explained by the amphiphilic structure of 1-Hexadecanoyl-sn-glycerol. The molecule contains a hydrophobic palmitoyl chain and a hydrophilic glycerol moiety, enabling interaction with bacterial membranes. Similar compounds have been reported to integrate into membrane phospholipid bilayers, resulting in increased membrane fluidity, destabilization, and eventual cell death (Desbois and Smith, 2010). Kabara et al. (1972) were among the first to demonstrate that fatty acid derivatives possess potent antimicrobial properties through direct membrane disruption. Likewise, Bergsson et al. (2001) reported that monoglycerides exert antimicrobial effects by damaging membrane integrity and causing leakage of intracellular contents. The MIC and MBC results further supported the antibacterial efficacy of the isolated compound. The low MIC values recorded against MRSA and VRSA indicate that Gram-positive bacteria are particularly sensitive to 1-Hexadecanoyl-sn-glycerol. Furthermore, the MBC/MIC ratio of approximately 2 suggests a bactericidal mode of action rather than a merely bacteriostatic effect. According to French (2006), compounds exhibiting low MBC/MIC ratios are generally considered effective bactericidal agents. The ability of 1-Hexadecanoyl-sn-glycerol to completely eliminate bacterial cells at relatively low concentrations highlights its therapeutic potential against resistant pathogens.

Biofilm formation is recognized as one of the most important virulence factors contributing to chronic and recurrent infections. Biofilms provide bacterial cells with enhanced protection against antibiotics, host immune responses, and environmental stress (Costerton et al., 1999). In the present study, 1-Hexadecanoyl-sn-glycerol significantly inhibited biofilm formation in all tested bacterial species. The highest inhibition was observed against MRSA and VRSA, indicating that the compound effectively interferes with biofilm development in Gram-positive bacteria. Similar observations have been reported by

Hall-Stoodley et al. (2004), who emphasized that disruption of bacterial adhesion and extracellular polymeric substance production is a critical strategy for controlling biofilm-associated infections.

The antibiofilm activity of 1-Hexadecanoyl-sn-glycerol may be associated with its ability to interfere with bacterial surface attachment and membrane-associated signaling pathways. Fatty acid-derived molecules have been reported to disrupt quorum sensing systems and inhibit the synthesis of extracellular polymeric substances necessary for biofilm maturation (Nazzaro et al., 2013). The concentration-dependent reduction in biofilm biomass observed in the present study suggests that the isolated compound may interfere with early stages of biofilm establishment as well as subsequent biofilm development.

To further elucidate the mechanism of antibacterial action, membrane integrity assays were performed by measuring the leakage of intracellular nucleic acids and proteins. Treatment with 1-Hexadecanoyl-sn-glycerol resulted in a marked increase in extracellular absorbance at 260 nm and 280 nm, indicating substantial release of intracellular constituents. These findings provide direct evidence that the compound compromises bacterial membrane integrity. Similar membrane-disruptive effects have been reported for antimicrobial fatty acids and monoglycerides by Desbois and Smith (2010) and Bergsson et al. (2001). The release of nucleic acids and proteins following treatment strongly supports membrane permeabilization as a primary mechanism of bacterial killing.

The membrane-targeting mode of action observed in this study may offer significant advantages over conventional antibiotics. Most antibiotic resistance mechanisms involve modification of intracellular targets, enzymatic degradation, or active efflux systems. In contrast, compounds that directly disrupt membrane integrity are generally less susceptible to resistance development because they target fundamental structural components essential for bacterial survival (Epand and Epand, 2009). Therefore, the ability of 1-Hexadecanoyl-sn-glycerol to damage bacterial membranes may contribute to its effectiveness against MDR pathogens.

Overall, the present study demonstrates that 1-Hexadecanoyl-sn-glycerol possesses significant antibacterial and antibiofilm activities against clinically important multidrug-resistant pathogens. The observed biological activities appear to be mediated primarily through disruption of bacterial membrane integrity, leading to leakage of intracellular constituents and inhibition of biofilm formation. These findings highlight the potential of this naturally derived monoglyceride as a promising candidate for the development of novel antimicrobial formulations aimed at combating antibiotic-resistant bacterial infections.

5.0 Conclusion

1-Hexadecanoyl-sn-glycerol exhibited significant antibacterial activity against multidrug-resistant clinical pathogens, namely MRSA, VRSA, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The compound demonstrated promising inhibitory effects with low MIC and MBC values, indicating potent bactericidal activity. Furthermore, 1-Hexadecanoyl-sn-glycerol effectively inhibited biofilm formation in all tested pathogens, highlighting its potential to combat biofilm-associated infections that are often resistant to conventional antibiotic therapy. Membrane integrity assays revealed substantial leakage of intracellular nucleic acids and proteins following treatment, suggesting that membrane disruption constitutes the primary mechanism underlying its antimicrobial action.

Funding

The authors declare that no specific funding was received for this research from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgements

The authors express their sincere gratitude to the Management of Priyadarshini Degree and P.G., College, Kothagudem for providing laboratory facilities and the necessary infrastructure to carry out this research work. The author also acknowledge the technical assistance provided during the experimental studies.

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