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Mohammed Abu Sayeed

sayeed@iiuc.ac.bd

International Islamic University Chittagong

Mohammad Arman

International Islamic University Chittagong

Israt Jahan

International Islamic University Chittagong

Md. Abdul Mojid Mondol

Bangladesh Open University

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ORIGINAL ARTICLE

BIOACTIVE SECONDARY METABOLITES FROM MARINE-DERIVED STREPTOMYCES

Mohammed Abu Sayeed*[1,2], Mohammad Arman[1], Israt Jahan[1], Md. Abdul Mojid Mondol[3]

[1]Department of Pharmacy, Faculty of Science and Engineering, International Islamic University Chittagong, Kumira, Chattogram-4318, Bangladesh
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh
[3]School of Science and Technology, Bangladesh Open University. Board Bazar, Gazipur-1705, Bangladesh

Corresponding Author: Mohammed Abu Sayeed
Department of Pharmacy, Faculty of Science and Engineering, International Islamic University Chittagong, Kumira, Chattogram-4318, Bangladesh
Tel.: (+88-01676419435); Fax: +8802334461930); E-mail: sayeed@iiuc@ac.bd
ORCID: 0000-0003-3326-0979

Running title: Novel Streptomyces Antimicrobial Against MDR Pathogens.

ABSTRACT
Actinomycetes are prolific producers of secondary metabolites with substantial pharmaceutical and industrial significance. Genera such as Actinoplanes, Streptomyces, Saccharopolyspora, Amycolatopsis, and Micromonospora are well recognized for their ability to produce diverse bioactive compounds. The increasing prevalence of multidrug-resistant (MDR) pathogens has intensified the search for novel antimicrobial agents, particularly from underexplored ecological niches. Marine environments represent a promising reservoir of actinomycetes capable of synthesizing structurally diverse and biologically potent secondary metabolites. This study aimed to isolate and characterize a novel antimicrobial metabolite from a marine-derived Streptomyces strain and evaluate its antibacterial potential against a multidrug-resistant pathogen.
A marine-derived Streptomyces strain isolated from sediment was cultured, and its secondary metabolites were extracted and purified using chromatographic techniques. The isolated compound, designated Lovfung 1, was structurally elucidated using high-resolution mass spectrometry and comprehensive one- and two-dimensional nuclear magnetic resonance (NMR) spectroscopic analyses. Its antibacterial activity was evaluated against a multidrug-resistant Klebsiella sp. using the disc diffusion method. Lovfung 1 was successfully isolated and structurally characterized based on high-resolution mass spectrometry and 1D/2D NMR data. The compound exhibited notable antibacterial activity against the tested multidrug-resistant Klebsiella sp., producing a 15 mm zone of inhibition at 100 µg/disc. The findings demonstrate the potential of marine-derived Streptomyces as a source of bioactive secondary metabolites with antibacterial activity against drug-resistant pathogens. Further studies, including structure–activity relationship investigations, in-depth mechanism-of-action studies, broader-spectrum efficacy assessment, minimum inhibitory concentration determination, and toxicity profiling, are warranted to establish the therapeutic potential of Lovfung 1.

Keywords: antibiotics; actinomycetes; multidrug resistance; Lovfung 1; marine sediment.

INTRODUCTION

Background

The discovery of penicillin by Florey and Chain in the 1940s, derived from the mold *Penicillium notatum*, marked the beginning of the antibiotic revolution and is widely regarded as one of the most transformative milestones in medical history. Its remarkable capacity to treat previously fatal bacterial infections earned it the reputation of a "miracle drug." However, within just a few years of its clinical use, resistant bacterial strains began to emerge, and by the 1950s, the therapeutic breakthroughs achieved in the earlier decade were already being undermined by the rise of penicillin resistance (Neu, 1992; Rehm, 2008). Regretfully, resistance to almost all developed antibiotics has finally been observed (Control, Prevention %J Atlanta, & Prevention, 2013).

The ability of microorganisms to withstand exposure to medications that were formerly successful against them is known as antimicrobial resistance (AMR). Nearly 70% of harmful bacterial species are thought to have become resistant to at least one class of antibiotics that are currently on the market. Infections brought on by these resistant organisms increase the risk of complications and death, lengthens sickness, and raise treatment expenses (Katz, Mueller, Polyakov, & Weinstock, 2006). With millions of fatalities per year, AMR is acknowledged as a serious public health emergency worldwide, with the burden being greatest in low- and middle-income nations. In nations such as Bangladesh, the widespread availability and inappropriate use of antimicrobial drugs in both human and veterinary medicine have accelerated the spread of resistance (Shankar & Balasubramanium, 2014). In many cases, antibiotics are dispensed without prescription or proper diagnostic justification, greatly aiding in the development of resistant strains.

A Gram-positive organism of considerable clinical importance, Staphylococcus aureus leads to a wide range of infections, from mild soft tissue and skin lesions, including folliculitis, to severe systemic illnesses such as toxic shock syndrome and bacteremia. Over the past two decades, infections caused by *S. aureus* have become increasingly difficult and expensive to manage due to rising levels of antimicrobial resistance (**Lowy, 1998**). The multidrug resistant *S. aureus* (MDRSA) and Methicillin resistant S. *aureus* (MRSA) bacteria are especially concerning since they are resistant to almost all β-lactam antibiotics as well as several other widely used substances. Methicillin resistant S. aureus (MRSA) and multidrug resistant *S. aureus* (MDRSA) are major concerns (Gordon & Lowy, 2008). In some reports, more than 70% of clinical *S. aureus* isolates resistant to methicillin [or oxacillin] display cross-resistance to multiple antibiotic classes, resulting in higher rates of morbidity and mortality assessed to methicillin-sensitive *S. aureus* (MSSA) infections (**Moellering Jr, 2012**). The U.S. Centres for Disease Control and Prevention (CDC) estimate that every year, about two million Americans contract antibiotic-resistant illnesses, results in minimum 23,000 mortality that are directly caused by resistant microorganisms (**Klevens *et al.*, 2007**). Similarly, across Europe, MRSA and other "superbugs" are responsible for an estimated 400,000 hospital-acquired infections annually, causing around 25,000 deaths and imposing an economic burden exceeding €1.5 billion per year.

Methicillin resistant *S. aureus* (MRSA) remains a major global public health concern and is among the most prevalent antibiotic-resistant pathogens identified worldwide. In certain regions, its prevalence has been reported to reach nearly 100% (**Lagamayo, 2008**). The World Health Organisation (WHO) listed MRSA on its 2017 global importance list of antibiotic-resistant bacteria, giving it a high priority level for the development of novel and efficient antimicrobial medicines due to its clinical relevance and lack of available treatments (**Tacconelli, 2017**). Genetic mutations and adaptive mechanisms enable bacteria to develop resistance to antibiotics making it more challenging to treat the infections they produce. According to the World Health Organization (WHO), drug-resistant diseases are responsible for approximately 700,000 deaths each year, including around 230,000 fatalities due to multidrug resistant tuberculosis. A growing number of infections affecting the urinary and respiratory tracts, as well as several sexually transmitted infections, are becoming refractory to available therapies. Lower respiratory tract infections continue to be the most common cause of mortality globally among infectious illnesses, claiming an estimated three million lives annually, with the highest burden observed in low-income countries.

The pace at which new medications that are effective against methicillin resistant *S. aureus* (MRSA) are discovered has drastically decreased in recent years, even as resistance continues to accumulate at an alarming pace. Several factors have contributed to this stagnation in antibiotic innovation. A key reason is the withdrawal of major pharmaceutical companies from antibacterial research and development, largely because short-duration antibiotic treatments are less profitable than drugs used for chronic conditions **(Projan, 2003**). Moreover, the limited success of recent discovery programs underscores the inherent challenges of developing compounds active against MRSA, which possesses unique genetic and biochemical traits distinguishing it from other *S. aureus* strains. Consequently, there is an urgent global need to pursue innovative strategies for the identification of new anti-MRSA agents to safeguard public health and save millions of lives.

Roughly 70% of the antibiotics in clinical use today were originally discovered through systematic screening of compounds derived from microbial fermentation. Among microorganisms, actinomycetes are renowned as an inexhaustible reservoir of bioactive metabolites, particularly antibiotics. Most antimicrobial agents used in medicine were first isolated from members of this group, with Streptomyces species contributing the majority. These bacteria have produced nearly all major antibiotic classes currently prescribed, including β-lactams, tetracyclines, macrolides, aminoglycosides, and glycopeptides (Solanki, Khanna, & Lal, 2008). However, over recent decades, the remarkable efficacy of these therapeutic agents has been increasingly compromised by the rapid evolution of resistance among pathogenic bacteria, threatening their continued clinical utility

Actinomycetes represent one of the most prolific and versatile microbial groups capable of synthesizing a vast array of secondary metabolites with significant industrial and pharmaceutical relevance. Over past decades, numerous species have been isolated and extensively characterized from terrestrial environments, particularly soil. More recently, actinomycete populations have also been identified within previously unexplored marine ecosystems. With their antibacterial, antifungal, anticancer, insecticidal, and enzyme-inhibitory qualities, these marine-derived strains have shown themselves to be abundant sources of structurally varied and physiologically active chemicals.

The unique physicochemical characteristics of the deep-sea habitat are another important factor. It is believed that marine actinomycetes have distinct physiological and biochemical characteristics from their terrestrial counterparts. This divergence likely results from the extreme ecological pressures of their habitats such as intense competition for space, limited nutrient availability, predation, variations in light and oxygen levels, and high hydrostatic pressure (Casertano, Menna, & Imperatore, 2020). As a result, marine actinomycetes' secondary metabolites frequently exhibit unique structural features not observed in land-derived species. These unique chemical architectures provide valuable scaffolds to produce novel therapeutic substances that are capable of countering resistant of drug pathogens.

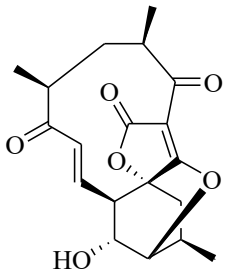
1Bioactive secondary metabolites from marine actinomycetes act against MDR

To date, more than 350 genera have been identified within this group, which is among the biggest known bacterial phyla. These microorganisms are extensively available across both terrestrial and aquatic environments, reflecting their ecological adaptability. Most actinomycetes -particularly those belonging to the genus Streptomyces- are saprophytic, thriving in soil where they show a vital function in organic matter decomposition. However, they are also encountered in marine and freshwater environments, as well as in the air. Soil populations of actinomycetes generally range between 10^6 and 10^9 cells per gram, with Streptomyces species constituting more than 95% of the isolates typically recovered (**Barka *et al.*, 2016**). While many actinomycetes are non-pathogenic and even beneficial to plants and animals, certain species are recognized as opportunistic or pathogenic organisms of medical and agricultural relevance.

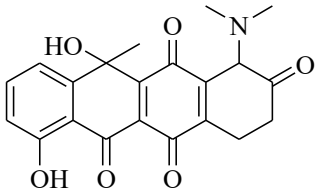
About half of all known secondary metabolites are produced by actinomycetes, significant families of prokaryotes. Owing to their exceptional capability to synthesize novel bioactive compounds, extensive research over the past five decades has focused on isolating new actinomycete strains from terrestrial environments for drug discovery purposes. Nevertheless, the rate at which novel metabolites from terrestrial isolates have been discovered has decreased recently, while the re-isolation of already known compounds has become increasingly common.

To address this challenge, attention has shifted toward exploring actinomycetes from previously untapped or underexplored ecological niches in search of new secondary metabolites effective against multidrug resistant pathogens. Marine actinomycetes, in particular, have demonstrated remarkable chemical diversity and often yield bioactive molecules with greater structural novelty and potency compared to their terrestrial counterparts. The pursuit of such metabolites is driven primarily by the urgent need to develop new therapeutics capable of combating resistant microbial infections that no longer respond to existing antibiotics. Strong efficacy against multidrug resistant infections has been demonstrated by a number of antimicrobial agents derived from marine actinomycetes; a few noteworthy examples are outlined below:

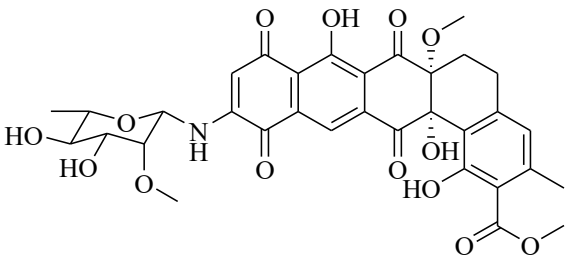
The marine actinobacterium Verrucosispora sp. produces the unique polycyclic polyketide antibiotic abyssomicin C [1]. This substance has strong antibacterial properties versus a variety of Gram positive bacteria, including clinical isolates of *S. aureus* that are resistant to vancomycin and other drugs. (**Bister *et al.*, 2004**). Another marine-derived actinomycete, *S. psammoticus*, has been reported to produce the antibiotic SBR-22 [2], which has potent anti-methicillin resistant *S. aureus* (MRSA) inhibitory actions (Sujatha, Raju, & Ramana, 2005). Methicillin resistant *S. aureus* and vancomycin-resistant *Enterococcus fecium* are two examples of human pathogenic bacteria that are inhibited in their development by Marinomycin, which was purified from the marine actinomycete strain "Marinispora" [3,20].



1



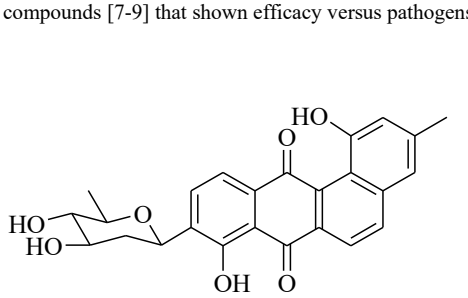
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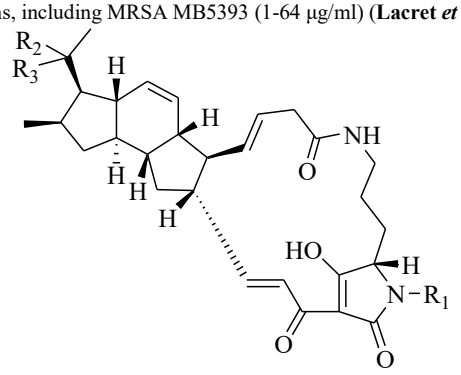
4

Salinispora arenicola, an obligatory marine actinomycete, produces arenimycin [4], a recently discovered antibiotic that is a member of the benzonaphthacene quinone family. The first member of this structural class to be identified from *S. arenicola* is this chemical. Arenimycin exhibits strong antibacterial action against *methicillin resistant S. aureus* (MRSA) that is resistant to both methicillin and rifampin. With minimum inhibitory concentrations (MICs) usually less than 1 µg/mL, it also has potent inhibitory effects against a variety of drug-resistant Staphylococcus strains and other Gram positive human infections (Asolkar, Kirkland, Jensen, & Fenical, 2010).

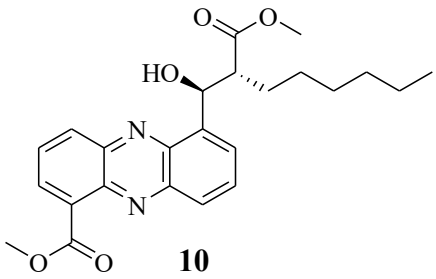
Methicillin resistant *S. aureus* (MRSA) strain ATCC 33591 has been shown to be significantly inhibited by the antibacterial chemical coumarin-6-ol, 3,4-dihydro-4,4,5,7-tetramethyl [5], which is produced from Streptomyces strain VITAK1. Its IC_{50} value is 40 µg/mL. Additionally, this compound demonstrated broad-spectrum antibacterial potentials for bacterial pathogens of both Gram positive and Gram negative types (Abirami, Gopal, Kannabiran, & Microbiology, 2015). Similarly, Streptomyces sp. SCSIO 11594, which was isolated from the South China Sea at a depth of 2,403 meters, demonstrated substantial inhibition against methicillin resistant Staphylococcus epidermidis [MRSE] strain shhs-E1 with a minimum inhibitory concentration (MIC) of 16 µg/mL (**Song *et al.*, 2015**). *S. zhaozhouensis* CA-185989 (Equatorial Guinea) generated three novel compounds [7-9] that shown efficacy versus pathogens, including MRSA MB5393 (1-64 µg/ml) (**Lacret *et al.*, 2014**).



6

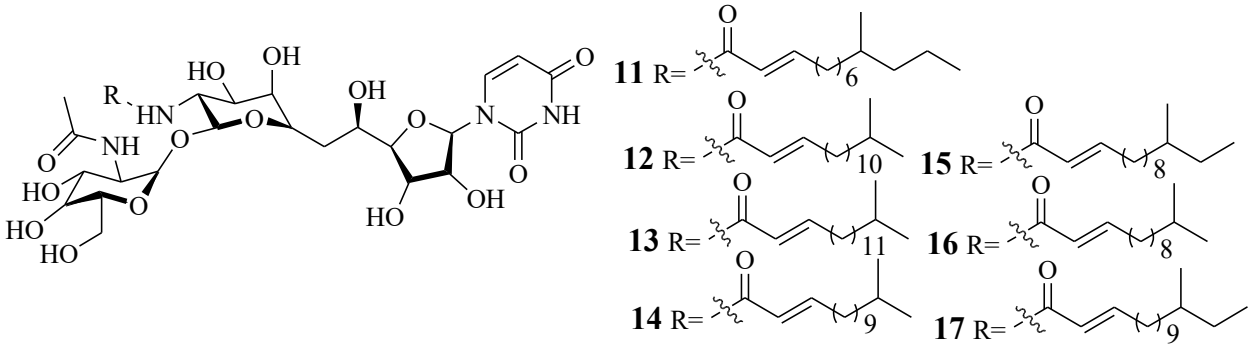


7 $R_1=R_2=R_3=H$
8 $R_1=CH_3$, $R_2=R_3=H$
9 $R_1=CH_3$, $R_2=R_3=O$

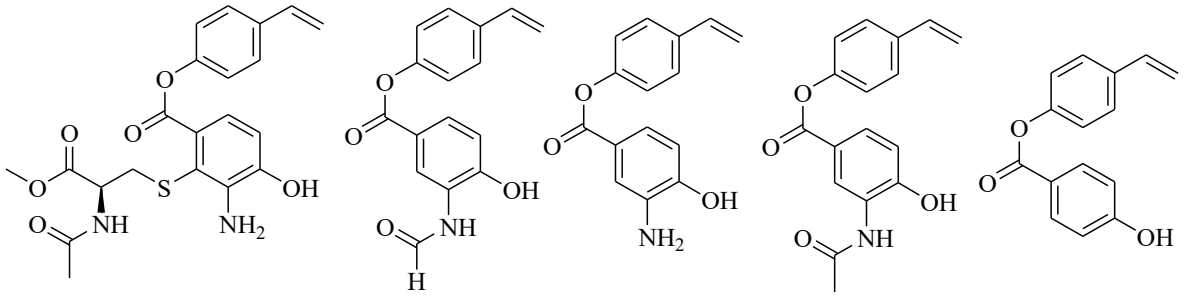


10

Streptomyces sp. 182SMLY [a marine actinomycete] was used to isolate [-]-Streptophenazine B [10], which demonstrated effectiveness in inhibiting the growth of methicillin resistant *S. aureus* with a MIC value of 4.2 µg/mL (Liang et al., 2017). Antimicrobial compounds [new tunicamycin E [11] and other identified compounds B, X, A, D2, C, and C2 [12–17] generated by *S. xinghaiensis* SCSIO S15077 (3536 m) isolated from South China Sea sediment were effective against a fluconazole-resistant strain of *Candida albicans* ATCC 96901 (4.0–32 µg/mL) and other test pathogens (**Zhang *et al.*, 2020**).



Five bagremycin analogues, including two new bagremycins F [**18**] and G [**19**] and three known bagremycins A [**20**], B [**21**], and E [**22**] were purified from *Streptomyces* sp. ZZ745 [also a marine actinomycete]. All bagremycins [**18-22**] showed MIC against MRSA, *E. coli*, and *C. albicans* in range of 67-176 μM.



18 **19** **20** **21** **22**

Four newly identified medermycin-type naphthoquinones—strepoxepinmycins A–D [23–26]—along with one previously known analogue, medermycin, were isolated from Streptomyces sp. XMA39. All four novel compounds displayed pronounced antibacterial activity against Escherichia coli and methicillin resistant *S. aureus* (MRSA), as well as antifungal effects against Candida albicans. Their minimum inhibitory concentrations (MICs) ranged from 0.25 to 15 μg/mL, indicating broad-spectrum potency against both bacterial and fungal pathogens (**Jiang *et al.*, 2018**).

From the above discussion, marine actinomycetes produce diversified bioactive compounds act against multidrug resistance pathogens. For this reason, marine sediment sample was used in this research to find out bioactive compounds. This investigation aims to determine structure of the novel bioactive agents by spectroscopic techniques and to study activity against drug resistance pathogens.

MATERIALS AND METHODS

Experimental

PDA (HiMedia, India), n-hexane (Daejung Chemicals and Metals Company Ltd, Korea), ethyl acetate (Scharlau, Spain), methanol (Scharlau, Spain), sterile filter paper disc (BioMaxima S.A., Poland), BaCl2.H2O (Merck, Germany), 13 standard antibiotics (HiMedia, India), and Mueller Hinton Agar (HiMedia, India) were purchased from regional vendors. A vertical autoclave (Model: LVA-202, Labocon, UK), a horizontal laminar airflow cabinet (Model: LLFH-204, Labocon, UK), and a rotary evaporator (Model: HS-2005S-N, Hahnshin S&T Co., Ltd., Korea) were used for sterilisation processes, aseptic operations, and solvent evaporation. To guarantee the purity and dependability of the experimental results, all of the solvents used in this investigation were either analytical grade or freshly distilled before use.

Purification of Streptomyces NRS01 strain

The *Streptomyces* NRS01 strain was purified from a sediment sample sourced from Naf River, Teknaf, Cox's Bazar, Bangladesh during an expedition in 2009 by serial dilution technique. In brief, one gram of the sediment sample was dissolved in 5 ml sterilized distilled water in aseptic conditions, mixed well and kept undisturbed for 10 min. 100 μL supernatant was spread on the previously sterilized Czapek Dox agar (Composition: soluble starch10 g, casein 0.3 g, KNO_3 2 g, $MgSO_4.7H_2O$ 0.05g, K_2HPO_4 2g, NaCl 2 g, $CaCO_3$ 0.02 g, $FeSO_4.7H_2O$ 0.01 g, Agar powder 30g and H_2O 1liter) in 150 mm Petri dish (Figure 1). After rappig the plate with parafilm were incubated at 30°C until the sporulation of *Streptomyces* NRS01 strain colonies was appeared. The strain NRSS01 (Figure 2) was isolated from culture plate by repeatedly streaking on Czapek Dox agar medium and identified as genus *Streptomyces* based on morphology.



Figure 1 Sediment sample culture plate



Figure 2 Isolated *Streptomyces* NRS01 strain

Seed and Mass Cultures of the Strain NRSO1

Seed and production cultures were prepared in a modified Czapek Dox broth medium containing 2.5% salt. The production media and the seed culture medium had the same pH and content. Before inoculation, a 500 mL Erlenmeyer flask was filled with a 200 mL aliquot of the medium, which had been sterilized beforehand. After being extracted from an agar plate, a single colony of Streptomyces strain NRS01 was aseptically added to the flask and cultured for two days at 30 °C under static circumstances. The seed culture was aseptically inoculated with an aliquot [0.2% v/v) into a 2 L flask [a total of 20 flasks] that contained 150 ml of sterilized Czapek Dox broth medium. After seven days of incubation under the identical circumstances as the seed culture, the production culture was harvested.

Solvent Extraction and Isolation

The mass culture broth (3 L) (Fig. 3) was filtered, and the filtrate was extracted with solvent EtOAc (1 × 3 L). The EtOAc layer was concentrated to dryness using rotary evaporators *in vacuo* at 40 °C. The residual suspension (1 g) was subjected to Sephadex LH-20 open column chromatography with MeOH-EtOAc [v/v] (1:1) as eluant. The elute was collected as three fractions: F-1, F-2 and F-3. The fraction F-1 was subjected to PTLC purification with solvent system-MeOH: EtOAc: n-hexane=0.5 : 2: 3 to yield pure compounds **1** (23 mg).

Collection of test pathogens

Lovofung 1 (1)'s antibacterial efficacy was evaluated against a clinical isolate of Klebsiella sp. that was resistant to many drugs. The Rajshahi Medical College's Microbiology Laboratory in Rajshahi, Bangladesh, provided the test strain. Prior to testing, this isolate was shown to be resistant to many kinds of antibiotics and came from a clinical sample taken from an infected patient.

Preparation of inoculums of test pathogens

The test pathogens were scattered onto sterile Mueller–Hinton agar plates, which were prepared as per formulation and incubated for 24 hours at 37 °C to create the seed culture. The inoculum was prepared by suspending bacterial colonies in sterile physiological saline solution [0.9% NaCl, w/v] from the resultant fresh culture. Then, by adding more colonies or saline as necessary, the turbidity of each bacterial solution was brought to the 0.5 McFarland standard, or around 1.5×10^8 CFU/mL (**Kiehlbauch *et al.*, 2000**). This inoculum was used to study potential of Lovfung 1.

Antimicrobial potential test of 1

The disc diffusion technique was used to examine the spice extracts' antibacterial activity (Jorgensen & Turnidge, 2015). To guarantee equal grass development, a sterile cotton swab was used to uniformly spread a 50 µL aliquot of the standardized bacterial solution (1.5×10^8 CFU/mL) onto Mueller–Hinton agar (MHA) plates. Lovofung 1 [1] was dissolved in a mixture of ethyl acetate and methanol to obtain a concentration such that 10 µL of the solution contained 200 µg of the compound. 10 µL of this solution was used to saturate sterile filter-paper discs (6 mm in diameter), which were then carefully put on the infected agar surface after being thoroughly dried under laminar airflow. A standard ruler was used to measure the widths of the inhibition zones (Figure 4) in mm after the plates had been incubated for 24 hours at 37 °C. Blank discs were utilized as negative controls, while thirteen commercial antibiotic discs were used as positive controls.



Figure 3 Broth culture flasks of the Strain NRSO1

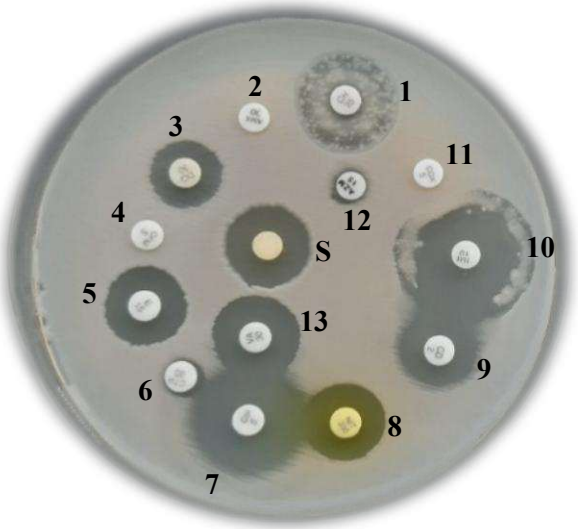


Figure 4 Activity test plate against *Staphylococcus aureus* S for Sample Lovfung 1 (100 µg)

RESULTS AND DISCUSSION

Lovfung (1) was an amorphous, yellowish solid. After a thorough examination of its combined mass spectrum [m/z 453.9 $[M + H]^+$], 1D and 2D NMR data, that required 11 degrees of unsaturation, the chemical formula was identified as $C_{22}H_{25}NO_4$. 11 degrees of unsaturated were ascribed to nine double bonds and two rings. The ^{13}c nmr, ^{1}H NMR, DEPT, HSQC and HMBC spectra exhibited eight olefinic carbon signals [δ_C 91.66 – 134.36], one methylene carbon [δ_C 21.16], three oxygenated methine carbons (δ_C 68.66-75.43), two methyl carbons (δ_C 6.18 and 52.1) one of which was oxygenated, and three carbonyl carbon (δ_C 167.04-197.19) (Table 1). In the ^{1}H-NMR spectrum, characteristics signals for methyl (CH_3), methylene (CH_2), methoxy [OCH_3], hydroxy (OH), olefinic and amide (NH) protons were observed.

The planar structure of **1** (Figure 5) was determined by analysis of its ^{1}H-^{1}H COSY, HSQC and HMBC spectroscopic data. The protons bound to their respective carbons were assigned unambiguously via the HSQC spectrum interpretation (Table 1).

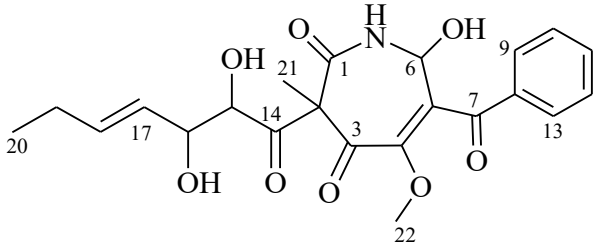


Figure 5 Planar structure of **1**

Analysis of ^{1}H-^{1}H COSY spectrum suggested two isolated spin systems: one between H-15 at δ_H 4.35 to H_3-20 at δ_H 0.89, and another between H-9 at δ_H 8.26 to H-11 at δ_H 7.69, and their connectivity with C-14 and C-8 (δ_C 133.90) explained by the HMBC correlations of H-15 with C-14 and H-9 with C-8, respectively (Figure 6). The connectivity of methoxy (OCH_3) group (δ_H 3.25, s) with olefinic carbon C-4 (δ_C 93.00) was easily determined as its protons showed HMBC correlation with C-4 (Fig. 2). Similarly, the remaining methyl group signal (δ_H 1.64, s, H_3-21) showed HMBC cross-peaks with C-2 (δ_C 112.05, quaternary carbon], C-3 [δ_C 197.19, keto carbon] and C-14 (δ_C 187.42, keto carbon). Amide proton (δ_H 9.96, brs, NH) showed HMBC correlations with C-1 (δ_C 167.04), C-5 (δ_C 91.66) and C-6 [δ_C 75.43). Finally, the structure of **1** was established as shown in the Fig. 2 and name lovfung **1**.

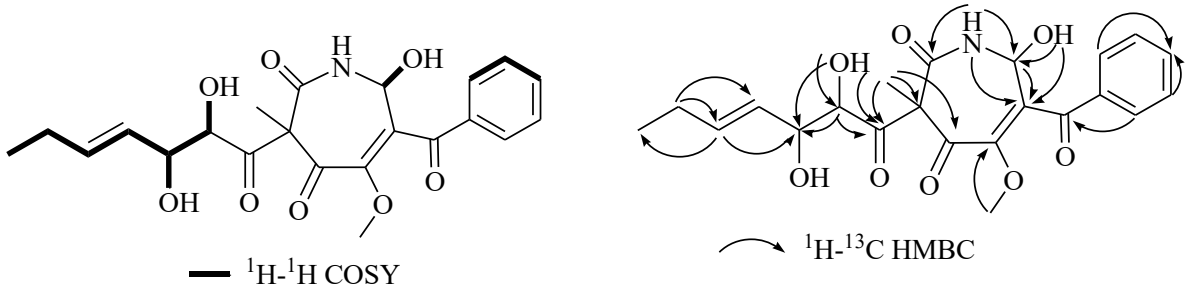


Figure 6 COSY and HMBC correlation for **1**

Table 1 ^{1}H and ^{13}C NMR data of 1 in DMSO-d_6.

Carbon No	[a]δ_C	[b]δ_H [*J* in Hz
1	167.04	
2	112.05	
3	197.19	
4	93.00	
5	91.66	
6	75.43	4.41 [d, *J* = 8.8 Hz, 1H]
7	196.94	
8	133.90	
9/13	130.79	8.26 [d, *J* = 7.7 Hz, 2H]
10/12	128.91	7.54 [t, *J* = 7.7 Hz, 2H]
11	134.36	7.69 [t, *J* = 7.7 Hz, 1H]
14	187.42	
15	72.43	4.35 [t, *J* = 5.0 Hz, 1H]
16	68.66	4.46 [dt, *J* = 8.8, 4.7 Hz, 1H]
17	129.54	5.39, m
18	134.25	5.43, m
19	21.16	2.02 [m, 2H]
20	14.62	0.89 [t, *J* = 7.5 Hz, 3H]
21	6.18	1.64 [s, 3H]
22	52.17	3.25 [s, 3H]
15-OH		4.90 [brd, *J* = 5.3 Hz, 1H]
16-OH		5.76 [brd, *J* = 5.5 Hz, 1H]
6-OH		6.26 [d, *J* = 9.1 Hz, 1H]
NH		9.96 [brs, 1H]
[a]Measured at 700 MHz; [b]Measured at 175 MHz		

Lovfung **1** and different 13 standard antibiotics were subjected to activity test against multidrug resistant *Klebsiella* sp. 5 out of 13 standard antibiotics did not demonstrate any potentiality against tested organisms (Table 2). Lovfung **1** showed 15 mm zone of inhibition against tested pathogen (Table 2).

Although more than 30000 human diseases have been described clinically, less than a third can be treated symptomatically, and a small percentage can be cured. Thus, there is an immediate need for new therapeutic agents to meet the unmet medical needs in clinical practice worldwide. Natural products have long been regarded as a rich source of lead compounds for drug discovery and development. Marine actinomycetes are a relatively new source of secondary metabolites, but they have yielded an impressive number of bioactive molecules during the last few years of intensive research.

S. aureus is the etiological agent of almost half of the hospital-acquired infections and causes about 100000 deaths in the United States every year. Moreover, hospital staff is regularly exposed to other Gram positive bacteria such as Enterococcus and Streptococcus species, as well as Gram negative Klebsiella, Escherichia, Enterobacter, Serratia, Citrobacter, Salmonella, and Pseudomonas aeruginosa. Hospital-acquired infections are caused by “nosocomial” microorganisms.

Over 60% of nosocomial sepsis episodes are caused by Gram negative bacteria [30]. Thus, there is a continuous need for novel anti-infective agents since there is an increasing resistance of microorganisms to almost all existing antibiotics. Therefore, to combat the ever-emerging clinical challenges posed by the constant emergence of multi-resistant pathogens, new classes of anti-infective agents must be developed and introduced into clinical practice on a regular basis.

Table 2 Antibacterial activity of **1** and standard antibiotics (1-13) against *Klebsiella* sp.

SN	**Tested samples**	**Zone of inhibition [in mm]**
S	Lovfung 1 [100 µg/disc]	15
	Standard antibiotics [µg/disc]	
1.	Cefradine [25 µg]	16
2.	Amoxycilline [30 µg]	-
3.	Doxycycline [30 µg]	11
4.	Cefixime [5 µg]	-
5.	Erythromycin [15 µg]	15
6.	Ceftriaxone [30 µg]	-
7.	Ciprofloxacin [5 µg]	20

8.	Nitrofurantoin [300 µg]	15
9.	Clindamycin [2 µg]	15
10.	Imipenem [10 µg]	20
11.	Cloxacillin [5 µg]	-
12.	Azithromycin [15µg]	-
13.	Vancomycin [30 µg	15
14.	Negative control [Sterile disc]	-
"-" Not active at tested concentration		

4-Bromophenol and bis [2 -ethylhexyl] phthalate was obtained from Nocardiopsis sp. strain SCA21 isolated from marine sediment presented activity against MRSA ATCC NR -46171 and MRSA ATCC -46071 was observed using the concentration of 15.62 and 7.81 µg/ mL as well as 125 and 15.62 µg / mL, respectively [31]. Desertomycin G was obtained from cultures of marine actinomycete *Streptomyces althioticus* MSM3 collected from samples of the intertidal seaweed Ulva sp. Desertomycin G demonstrated powerful antibiotic activity against Mycobacterium tuberculosis clinical isolates resistant to antibiotics used in clinical practice. In addition, desertomycin G also exhibited powerful antibiotic activities against several other clinically important Gram positive pathogens like Corynebacterium urealyticum, S. aureus, S. pneumoniae, S. pyogenes, Enterococcus faecium, Enterococcus faecalis, and Clostridium perfringens with range of 4-64 µg / mL [32]. Lovfung 1 showed antibacterial activity against MDR pathogen comparable with the literature data.

CONCLUSIONS

Marine actinomycetes create secondary metabolites with unique chemical structures that could serve as the foundation for the creation of novel medications. Actinomycetes retain an important place because of their diversity and demonstrated capacity to synthesize novel bioactive compounds that could serve as the foundation for the synthesis of new medications. They are also almost limitless sources of novel compounds with several therapeutic applications. Because this species produces between 50 and 55 % of the known antibiotics, the streptomycetes group of actinomycetes is regarded as economically significant. Since there is an increasing need to produce new and powerful treatment drugs to combat multidrug-resistant infections, the hunt for the discovery of rare and novel actinomycetes is especially crucial to finding new leads. Significant action was demonstrated by Lovfung 1 against Klebsiella sp. To establish Lovfung **1** as antibacterial agent further study such as MIC and toxicity are warranted.

List of abbreviations

1D: One dimension
2D: Two dimension
EtOAC: Ethyl acetate
MRSA: methicillin resistant *S. aureus*
PDA: Potato dextrose agar
NMR: Nuclear Magnetic Resonance
COSY: Correlation Spectroscopy
HSQC: Heteronuclear Single Quantum Coherence Spectroscopy
DEPT: Distortionless Enhancement by Polarization Transfer
HMBC: Heteronuclear Multiple Bond Correlation
DMSO: Dimethylsulfoxide
MeOH: Methanol

Author contributions

Conceptualization, M.A.S. and W.S.K.; methodology, O.K.R., W.S.K, H.M.S., and H.F. F.; software, W.S.K.; formal analysis, W.S.K. and B.M..; investigation, O.K.R., W.S.K, H.M.S., B.M., and H.F.F; data curation, O.K.R., W.S.K., and B.M.; writing— original draft preparation, O.K.R., W.S.K, H.M.S., B.M., and H.F.F.; writing—review and editing, O.K.R., W.S.K, H.M.S., B.M., and H.F.F; supervision, M.A.S;

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Consent for publication

The final draft of the manuscript was approved by all authors after they had examined the findings.

Authors' contributions

Conceptualization: Mohammed Abu Sayeed; Data curation: Md. Abdul Mojid Mondol; Formal analysis: Md. Abdul Mojid Mondol; Funding acquisition: Mohammed Abu Sayeed; Investigation: Mohammad Arman; Methodology: Md. Abdul Mojid Mondol; Project administration: Mohammed Abu Sayeed; Resources: Mohammed Abu Sayeed; Software: Mohammed Abu Sayeed; Supervision: Mohammed Abu Sayeed; Validation: Md. Abdul Mojid Mondol; Visualization: Israt Jahan; Writing–original draft: Mohammed Abu Sayeed; Writing–review & editing: Md. Abdul Mojid Mondol; All authors have read and agreed to the published version of the manuscript

Data availability

All relevant data can be found within the manuscript.

Competing interests

The authors declare that they have no competing interests.

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REFERENCES

Abirami, M., Gopal, J., Kannabiran, K. J. A. B., & Microbiology. (2015). Extraction and identification of antibacterial compound from marine Streptomyces sp. VITAK1 isolated from the coast of Andaman and Nicobar Islands, India. *51*(4), 406-410. https://doi.org/10.1134/S000368381504002X

Asolkar, R. N., Kirkland, T. N., Jensen, P. R., & Fenical, W. J. T. J. o. a. (2010). Arenimycin, an antibiotic effective against rifampin-and methicillin-resistant Staphylococcus aureus from the marine actinomycete Salinispora arenicola. *63*(1), 37-39. https://doi.org/10.1038/ja.2009.114

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.-P., . . . reviews, m. b. (2016). Taxonomy, physiology, and natural products of Actinobacteria. *80*(1), 1-43. https://doi.org/10.1128/mmbr.00019-15

Bister, B., Bischoff, D., Strobele, M., Riedlinger, J., Reicke, A., Wolter, F., . . . Sussmuth, R. D. J. A. C.-I. E. (2004). Abyssomicin CA polycyclic antibiotic from a marine Verrucosispora strain as an inhibitor of the p-aminobenzoic acid/tetrahydrofolate biosynthesis pathway. *43*(19), 2574-2582.

Casertano, M., Menna, M., & Imperatore, C. J. A. (2020). The ascidian-derived metabolites with antimicrobial properties. *9*(8), 510. https://doi.org/10.3390/antibiotics9080510

Control, C. f. D., Prevention %J Atlanta, G. C. f. D. C., & Prevention. (2013). Office of infectious disease antibiotic resistance threats in the United States, 2013.

Gordon, R. J., & Lowy, F. D. J. C. i. d. (2008). Pathogenesis of methicillin-resistant Staphylococcus aureus infection. *46*(Supplement_5), S350-S359. https://doi.org/10.1086/533591

Jiang, Y.-J., Zhang, D.-S., Zhang, H.-J., Li, J.-Q., Ding, W.-J., Xu, C.-D., & Ma, Z.-J. J. J. o. N. P. (2018). Medermycin-type naphthoquinones from the marine-derived Streptomyces sp. XMA39. *81*(9), 2120-2124. https://doi.org/10.1021/acs.jnatprod.8b00544

Jorgensen, J. H., & Turnidge, J. D. J. M. o. c. m. (2015). Susceptibility test methods: dilution and disk diffusion methods. 1253-1273. https://doi.org/10.1128/9781555817381.ch71

Katz, M. L., Mueller, L. V., Polyakov, M., & Weinstock, S. F. J. N. b. (2006). Where have all the antibiotic patents gone? *, 24*(12), 1529-1531. https://doi.org/10.1038/nbt1206-1529

Kiehlbauch, J. A., Hannett, G. E., Salfinger, M., Archinal, W., Monserrat, C., & Carlyn, C. J. J. o. c. m. (2000). Use of the National Committee for Clinical Laboratory Standards guidelines for disk diffusion susceptibility testing in New York state laboratories. *38*(9), 3341-3348.

Klevens, R. M., Morrison, M. A., Nadle, J., Petit, S., Gershman, K., Ray, S., . . . Townes, J. M. J. J. (2007). Invasive methicillin-resistant Staphylococcus aureus infections in the United States. *298*(15), 1763-1771.

Lacret, R., Oves-Costales, D., Gómez, C., Díaz, C., De la Cruz, M., Pérez-Victoria, I., . . . Reyes, F. J. M. d. (2014). New ikarugamycin derivatives with antifungal and antibacterial properties from Streptomyces zhaozhouensis. *13*(1), 128-140. https://doi.org/10.3390/md13010128

Lagamayo, E. N. J. A. j. o. i. c. (2008). Antimicrobial resistance in major pathogens of hospital-acquired pneumonia in Asian countries. *36*(4), S101-S108. https://doi.org/10.1016/j.ajic.2007.10.020

Liang, Y., Chen, L., Ye, X., Anjum, K., Lian, X.-Y., & Zhang, Z. J. N. P. R. (2017). New streptophenazines from marine Streptomyces sp. 182SMLY. *31*(4), 411-417. https://doi.org/10.1080/14786419.2016.1169419

Lowy, F. D. J. N. E. j. o. m. (1998). Staphylococcus aureus infections. *339*(8), 520-532. DOI: 10.1056/NEJM199808203390806

Moellering Jr, R. C. J. J. o. a. c. (2012). MRSA: the first half century. *67*(1), 4-11. https://doi.org/10.1093/jac/dkr437

Neu, H. C. J. S. (1992). The crisis in antibiotic resistance. *257*(5073), 1064-1073. DOI: 10.1126/science.257.5073.1064

Projan, S. J. J. C. o. i. m. (2003). Why is big Pharma getting out of antibacterial drug discovery? *, 6*(5), 427-430. https://doi.org/10.1016/j.mib.2003.08.003

Rehm, S. J. J. C. C. j. o. m. (2008). Staphylococcus aureus: the new adventures of a legendary pathogen. *75*(3), 177.

Shankar, P. R., & Balasubramanium, R. J. A. M. J. (2014). Antimicrobial resistance: global report on surveillance 2014. *7*(5), 237.

Solanki, R., Khanna, M., & Lal, R. J. I. j. o. m. (2008). Bioactive compounds from marine actinomycetes. *48*(4), 410-431. https://doi.org/10.1007/s12088-008-0052-z

Song, Y., Liu, G., Li, J., Huang, H., Zhang, X., Zhang, H., & Ju, J. J. M. D. (2015). Cytotoxic and antibacterial angucycline-and prodigiosin-analogues from the deep-sea derived Streptomyces sp. SCSIO 11594. *13*(3), 1304-1316. https://doi.org/10.3390/md13031304

Sujatha, P., Raju, K. B., & Ramana, T. J. M. r. (2005). Studies on a new marine streptomycete BT-408 producing polyketide antibiotic SBR-22 effective against methicillin resistant Staphylococcus aureus. *160*(2), 119-126. https://doi.org/10.1016/j.micres.2004.10.006

Tacconelli, E. (2017). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development.

Zhang, S., Gui, C., Shao, M., Kumar, P. S., Huang, H., & Ju, J. J. N. P. R. (2020). Antimicrobial tunicamycin derivatives from the deep sea-derived Streptomyces xinghaiensis SCSIO S15077. *34*(11), 1499-1504. https://doi.org/10.1080/14786419.2018.1493736