**Meditory**
**(The Journal of Medical Laboratory)**
**Volume 13 Issue 2 Page 172–186 December 2025**
**https://doi.org/10.33992/meditory.v13i2.4317**

# GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) PROFILING AND ANTIBACTERIAL ACTIVITY OF SECONDARY METABOLITES PRODUCED BY *Bacillus* sp. SA11 FROM MANGROVE SOIL

**Ni Kadek Widya Diari Ningsih, Anak Agung Gede Indraningrat[2*], Anak Agung Ayu Lila Paramasatiari [2], Daegeun Choe[3]**

[1]Study Program Medicine, Faculty of Medicine and Health Sciences, Warmadewa University, Jl. Terompong No. 24, Tanjung Bungkak, Denpasar, Bali, 80239, Indonesia
[2]Department of Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Warmadewa University, Jl. Terompong No. 24, Tanjung Bungkak, Denpasar, Bali, 80239, Indonesia
[3]FarmInUs, 1312 ho, 13F, 16 Maeheon-ro, Seocho District, Seoul, 06696, Republic of Korea

**Article History**
Received, 30th June 2025
Revised, 14th August 2025
Reviewed, 15th September 2025
Posted, 25th December 2025

**Editor**
I Wayan Karta

***Corresponding author**
Nama, e-mail:
Anak Agung Gede Indraningrat
indraningrat@warmadewa.ac.id

**Keywords**
Antibacterial, Bacterial, Infections, Bioprospecting

## Abstract

**Background:** Bacterial infections are a major global health concern, including in Indonesia. This has led to an increased effort to find new antibacterial compounds in natural resources. *Bacillus* sp. SA11, with reported antibacterial potential, was previously isolated from mangrove soil in Denpasar, Bali. This study further investigated its antibacterial activity.
**Methods:** Secondary metabolites were extracted from *Bacillus* sp. SA11 liquid culture using ethyl acetate. The extract was then screened for antibacterial activity against *Escherichia coli* ATCC 25922, *Streptococcus mutans* FNCC 0405, *Staphylococcus aureus* ATCC 25923, and *Klebsiella pneumoniae* ATCC 70060 using the Kirby-Bauer method (three replicates). The chemical composition of the extract was analysed via GC-MS.
**Results:** The ethyl acetate extract showed inhibition zones for *E. coli, S. mutans*, *S. aureus*, and *K. pneumoniae* of 11.7 ± 1.11 mm, 10.22 ± 1.5 mm, 8.7 ± 0.94 mm, and 7.2 ± 0.67 mm, respectively. GC/MS analysis identified 165 compounds, 20 of them have been previously linked to antibacterial activities. The five most abundant compounds are n-hexadecanoic acid (6.91%), 2-butoxyethyl acetate (6.3%), Bis(2-ethylhexyl) phthalate (4.45%), Phthalic acid, di(2-propylpentyl) ester (4.45%) and Benzene, 1,3,5-trimethyl- (Mesitylene).
**Conclusion:** These findings provide preliminary evidence that *Bacillus* sp. SA11, isolated from the mangrove ecosystem, has the potential to produce antibacterial compounds.

## Cite this Article

Ningsih NKWD, Indraningrat AAG, Paramasatiari AAAL, Choe D. Gas Chromatography-Mass Spectrometry (GC-MS) Profiling and Antibacterial Activity of Secondary Metabolites Produced by Bacillus sp. SA11 From Mangrove Soil. Meditory J Med Lab. 2025;13(2):172–186.

## INTRODUCTION

Bacterial infections are a pressing worldwide health issue, particularly in Indonesia (1). Current clinical practice heavily relies on antibiotic therapy, yet the widespread misuse and overuse of these drugs have led to a serious crisis: the development of antibiotic-resistant bacteria. This resistance can cause severe health complications and treatment failures. For example, *Streptococcus pneumoniae* has demonstrated resistance to Penicillin G. (2), and other notable resistant strains include multi-resistant *Mycobacterium tuberculosis* and Penicillin-Resistant *Pneumococci (3)*.

Infections caused by antibiotic-resistant bacteria pose a significant global challenge, leading to increased morbidity, higher treatment costs, and even fatalities (4). Previous studies in Indonesia have shown alarming rates of resistance, such as *Escherichia coli* exhibiting 90.27% resistance to ampicillin-sulbactam (5). Addressing antibiotic resistance requires a multi-faceted approach, including public education on responsible antibiotic use and, crucially, the exploration of novel antibacterial agents from natural sources. Continuous clinical and scientific innovation in drug development is essential to overcome this public health crisis (6).

Mangrove forest ecosystems thrive in the intertidal zones of muddy coastlines (7), are rich biodiversity hotspots. These unique environments are increasingly recognized as promising sources for novel antimicrobial compounds. Prior research has identified various bacteria from mangrove ecosystems, such as *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Enterobacter hormaechei*, *Klebsiella pneumoniae*, and *Enterococcus gallinarum*, exhibiting antibacterial activity against pathogens like *Vibrio alginolyticus* (8). Additionally, studies on endophytic bacteria isolated from mangroves have shown efficacy against *Staphylococcus aureus*, with clear zones of inhibition up to 13 mm (9).

In previous research, 22 out of 68 bacterial isolates that had antibacterial potential were found from the soil where the *Sonneratia alba* mangrove plant lives in the Ngurah Rai Mangrove Forest Park, Denpasar (10). Among the obtained isolates, one bacterial isolate encoded as *Bacillus* sp. SA11 showed antibacterial activity of 3 mm against *Streptococcus mutans* using the agar block method, which served as an initial indication of the antibacterial potential of *Bacillus* sp. SA11. However, the reported information has not fully described the antibacterial activity of *Bacillus* sp. SA11 because the previous antibacterial screening did not include chemical solvent extraction. The use of chemical solvents for extraction is intended to allow for a more thorough analysis of the antibacterial potential of *Bacillus* sp. SA11.

An earlier investigation on *Bacillus* sp SA11 did not explore the potential secondary metabolite compounds, as chemical solvents were not employed in the previous study (10). The present research concentrated on studying *Bacillus* sp. SA11, encompassing the extraction of active compounds using chemical solvent and screening for antibacterial activity. Therefore, the present study aimed to investigate whether *Bacillus* sp. SA11 could serve as a source for antibacterial compounds.

## MATERIALS AND METHODS

### Gram staining

The cell wall type and morphological features of *Bacillus* sp. SA11 were evaluated using the Gram staining technique (11). Subsequent microscopic observation of the bacterial cells was performed at 1000x magnification using a Leica DM750 light microscope (Leica Microsystems, Germany).

### Extraction of secondary metabolites from *Bacillus* sp. SA11

Briefly, cell colonies of *Bacillus* sp SA11 were grown in 100 mL of liquid ISP-2 media (4 g/L yeast extract, 10 g/L malt extract, 4 g/L dextrose, 20 g/L bacto agar) and incubated for 10 days on a shaker (150 rpm) (OHAUS, USA) at room temperature (12). Supernatant from pure culture isolates of *Bacillus* sp. SA11 was separated from the cell mass after 7 days. The cell-free supernatant was passed through a Whatman grade 1 filter paper (Cytiva, China) An equivalent volume of ethyl acetate pro-analysis was subsequently used for maceration of the filtrates (SMART-Lab, Indonesia). Finally, the liquid mixture was separated using a separating funnel. This extraction procedure was performed twice. The mixture was evaporated at 40 °C (Cole-Palmer, USA) to yield a thick extract, with its mass then determined using an analytical balance.

### Antibacterial Activity Test

Antibacterial activity was evaluated using the disc diffusion assay, also known as the Kirby-Bauer method. The procedure involved inoculating an agar plate with the target bacteria and then placing a disc impregnated with the test substance onto the surface. For the experiment, separate suspensions of *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* FNCC 0405, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC 700603 were prepared by adding 200 µL of each into sterile Luria-Bertani (LB) broth (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl, 20 g/L bacto agar), these suspensions were then uniformly spread across LB agar plates using sterile cotton swabs. Subsequently, three sterile 6 mm paper discs were impregnated with 20 µL of the *Bacillus* sp. SA11 ethyl acetate extract. The discs were positioned on the inoculated LB agar plates and left to incubate for 24 hours at 37°C (Memmert, Germany). For comparison, Levofloxacin was used as a positive control, while ethyl acetate served as a negative control. After incubation, the diameter of the clear zones around the discs was measured with a digital caliper (Vernier, USA) to determine the extract's inhibitory ability. The average diameter was then categorized into four levels of activity: weak (0-5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (> 20 mm)(13).

### GC-MS analysis

*Bacillus* sp. SA11 ethyl acetate extracts were submitted to the laboratory of Forensic Polda Bali to run the GC-MS analysis. The chemical constituents of the sample were analysed using an Agilent Technologies 7890B Gas Chromatograph coupled with an Agilent 5977B Mass Spectrometer Detector (MSD). Separation was performed on an Agilent HP-5ms Ultra Inert capillary column (30 m × 250 µm × 0.25 µm) with helium as the carrier gas at a constant flow rate of 2.9 mL/min. An injection volume of 1 µL was introduced in splitless mode with an injector temperature of 290°C. The oven temperature was initially held at 70°C for 5 minutes, then increased to 290°C at a rate of 10°C/min, and held for a

final 3 minutes. The MS was operated in electron ionization (EI) mode at 70 eV, with the transfer line, ion source, and quadrupole temperatures set at 290°C, 230°C, and 150°C, respectively. Data were acquired in scan mode over a mass range of m/z 50–550 after a 3-minute solvent delay. Compound identification was performed by comparing the mass spectra with the National Institute of Standards and Technology (NIST) and Wiley libraries. The resulting chromatogram was then interpreted by comparing the data with existing literature (11).

## RESULTS AND DISCUSSION

### Gram staining and observation

Morphological characteristics showed that the isolate is grouped as Gram-positive bacteria. Bacterial cells have a rod-shaped morphology as observed under a light microscope (Figure 1).

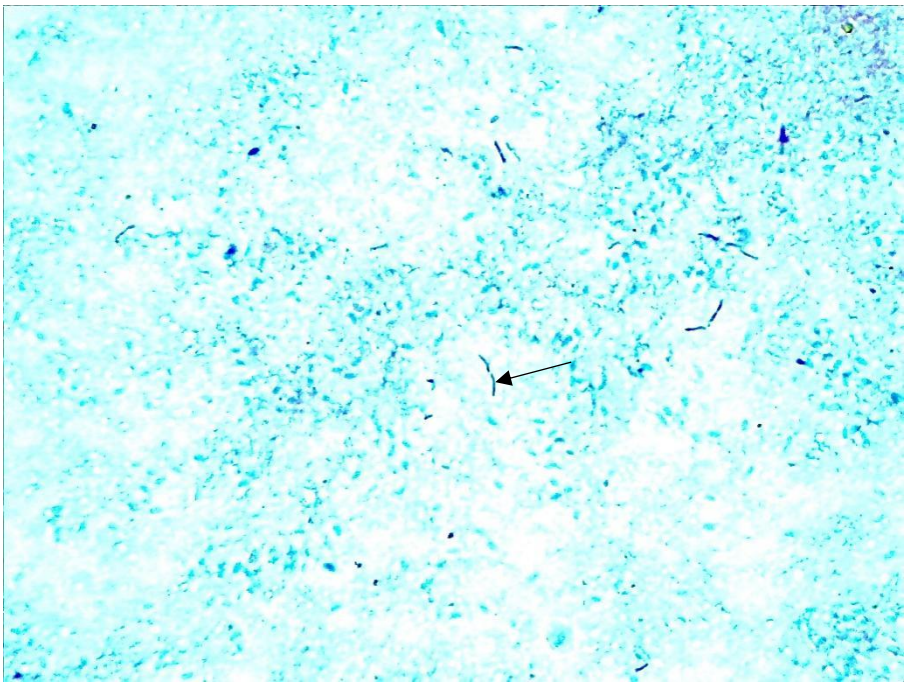

Figure 1. Gram staining and cells observation of *Bacillus sp.* SA11 (indicated by a black arrow) under light microscopy with 1000x magnification.

### Antibacterial Activity Screening

Extraction the supernatant of *Bacillus sp.* SA11 resulted in 0.743 gram of crude extracts. No antibacterial activity was detected in the negative control against any of the bacterial strains tested. Meanwhile, the ethyl acetate extract of *Bacillus* sp. SA11 was able to inhibit the test bacteria *S. aureus* ATCC 25923 with an average inhibitory zone diameter of 8.7±0.94mm, 10.22±1.5 mm against *S. mutans* FNCC 0405, 7.2±0.67 mm against *K. pneumoniae* ATCC 70060, and 11.7±1.11 mm against *E. coli* ATCC 25922 (Table 1, Figure 2). However, when compared with the antibacterial activity using levofloxacin, the observed antibacterial activity showed an inhibitory diameter approximately two to three times greater than that formed by the *Bacillus* sp. SA11 extract

Table 1. Antibacterial Activity Test Results

| Test Bacteria | Sample | Inhibition Zone Diameter (mm) ± SD | Interpretation |
|---|---|---|---|
| *S. aureus ATCC* 25923 | *Bacillus* sp. SA11 extract | 8.7±0.94 | Moderate |
| | Levofloxacin | 21.7±0.90 | Very strong |
| | ethyl Acetate | 0±0 | - |
| *E. coli ATCC* 25922 | *Bacillus* sp. SA11 extract | 11.7±1.11 | Strong |
| | Levofloxacin | 27.8±1.9 | Very strong |
| | Ethyl Acetate | 0±0 | - |
| *S. mutans* FNCC 0405 | *Bacillus* sp. SA11 extract | 10.22±1.5 | Strong |
| | Levofloxacin | 22±0.88 | Very strong |
| | Ethyl Acetate | 0±0 | - |
| *K. pneumoniae ATCC* 70060 | *Bacillus* sp. SA11 extract | 7.2±0.67 | Moderate |
| | Levofloxacin | 26.7±1.0 | Very strong |
| | Ethyl Acetate | 0±0 | - |

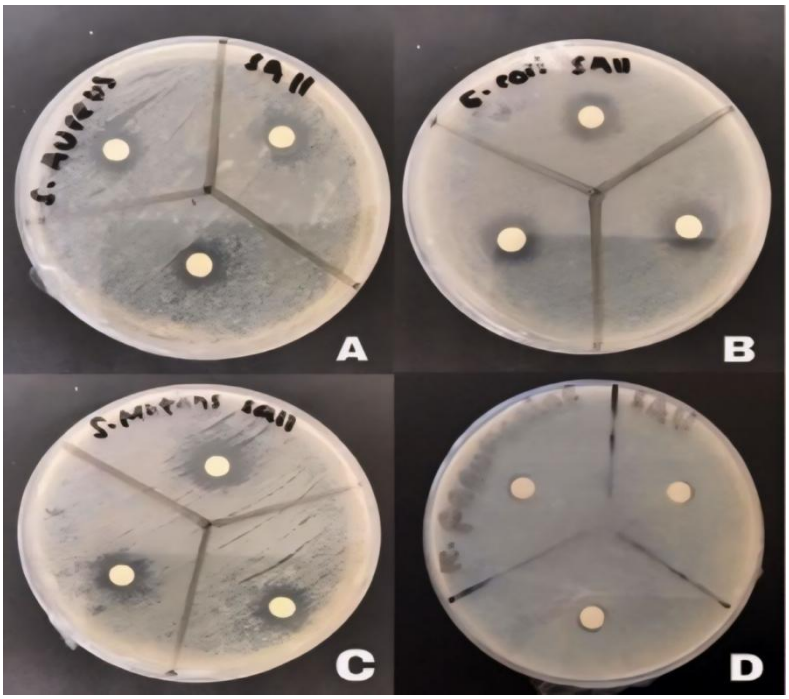


**Figure 2.** Antibacterial Activity Test of *Bacillus* sp. SA11 extract against test bacteria A: *S. aureus* ATCC 25923, B: *E. coli* ATCC 25922, C: *S. mutans* FNCC 0405, D: *K. pneumoniae* ATCC 700603.

The ethyl acetate extract of *Bacillus* sp. SA11 demonstrated varying degrees of antibacterial activity against the tested pathogens. Specifically, it exhibited moderate antibacterial activity against *Staphylococcus aureus* ATCC 25923, with an average inhibition zone diameter of 8.7 ± 0.94 mm. Stronger activity was observed against *Streptococcus mutans* FNCC 0405, yielding an average inhibition zone of 10.22 ± 1.5 mm, and against *Escherichia coli* ATCC 25922, which showed the most potent inhibition with an average zone of 11.7 ±

1.11 mm. In contrast, *Klebsiella pneumoniae* ATCC 70060 exhibited moderate susceptibility, with an average inhibition zone of 7.2 ± 0.67 mm.

These findings indicate that the *Bacillus* sp. SA11 extract possesses broad-spectrum antibacterial activity, effectively inhibiting the growth of both Gram-positive (*S. aureus*, *S. mutans*) and Gram-negative (*E. coli*, *K. pneumoniae*) bacteria (14). This broad efficacy aligns with previous reports of bioactive compound production by endophytic bacteria from mangrove plants, including halogenases, terpenoids, coumarins, alkaloids, peptides, and polyketides. Genera such as *Burkholderia*, *Bacillus*, and *Azospirillum*, isolated from *Sonneratia alba* in mangrove environments, have been identified as producers of antibacterial compounds (15). The observed antibacterial activity of *Bacillus* sp. SA11 may be attributed to the production of bacteriocins, protein compounds known for their bactericidal effects on other microorganisms (16, 17). Bacteriocins produced by *Bacillus* species typically induce damage to bacterial cell membranes, disrupting the controlled influx and efflux of essential cellular components and consequently impeding metabolic processes. This mechanism often involves the binding of bacteriocins to lipid components of the bacterial cell membrane, facilitated by their nonpolar nature (18). However, further research is required to confirm whether the antibacterial properties observed in *Bacillus* sp. SA11 are specifically associated with bacteriocins, as the precise quantity and nature of chemical compounds in the ethyl acetate extract were not quantified in this preliminary stage.

The differential antibacterial activity observed against the four test bacteria, with *E. coli* ATCC 25922 showing the highest susceptibility, could be explained by variations in bacterial defence mechanisms. The potential for *Bacillus* sp. SA11 to synthesize positively charged antibacterial compounds, such as bacteriocins, could be a mechanism for inhibiting Gram-negative bacteria. For example, the interaction of these compounds with the anionic outer membrane of *E. coli* could result in pronounced membrane disruption and suppress bacterial growth. Each bacterial species possesses unique cell wall compositions and resistance strategies, contributing to the varied responses to antibacterial agents (19).

**GC-MS analysis**

The GC-MS analysis detected 165 different peaks from the extract of *Bacillus sp.* SA11 (Figure 3. Of these 165 compounds, 20 of them highest percentages compounds have been corresponded with antibacterial activity based on literature studies (Table 2). The results of GC-MS analysis in Table 2 show that there are 12 groups of compounds that contain antibacterial, antioxidant, anticarcinogenic, and anticancer properties, as the compound benzeneacetamide, which has antibacterial, antioxidant, and anticancer activities. The three main compound components are compounds with large area percentages, namely n-hexadecanoic acid at 6.91%, 2-Butoxyethyl acetate at 6.3%, and Phthalic acid, di(2-propylpentyl) ester at 4.45%. Previous research on *Skimmia anquetilia* extract indicated that the presence of n-hexadecanoic acid has strong correlation with antibacterial activity (20). Similar findings on GC-MS analysis of Eucalyptus from Tunisia contained the component 2-butoxyethyl acetate, which was shown to have antimicrobial activity on *E. coli* and *Listeria monocytogenes* (21).

Intriguingly, the GC-MS profile also revealed the presence of two phthalate esters: bis(2-ethylhexyl) phthalate (DEHP) and phthalic acid, di(2-propylpentyl) ester. The detection of such compounds in biological extracts is a subject of considerable scientific debate. Phthalate esters, particularly DEHP, are high-production-volume chemicals used extensively as plasticizers in countless laboratory and industrial products, including

solvents, plasticware, and GC-MS components (22). Consequently, they are recognized as ubiquitous environmental and laboratory contaminants, and their detection in biological samples often arises from analytical artifacts rather than endogenous production (23).

Conversely, a growing body of literature reports the isolation of DEHP and other phthalates from various microorganisms and plants, where they are proposed to function as bioactive secondary metabolites (24). For instance, de novo biosynthesis of DEHP has been suggested in fungi and algae under controlled laboratory conditions designed to minimize external contamination (25). However, definitive proof of biosynthesis, such as through stable isotope labeling studies, remains rare in the literature. Therefore, while the presence of DEHP and phthalic acid, di(2-propylpentyl) ester in the *Bacillus* sp. SA11 extract is a notable finding, their origin cannot be definitively assigned as biosynthetic without further, rigorous investigation to exclude all potential sources of anthropogenic contamination.

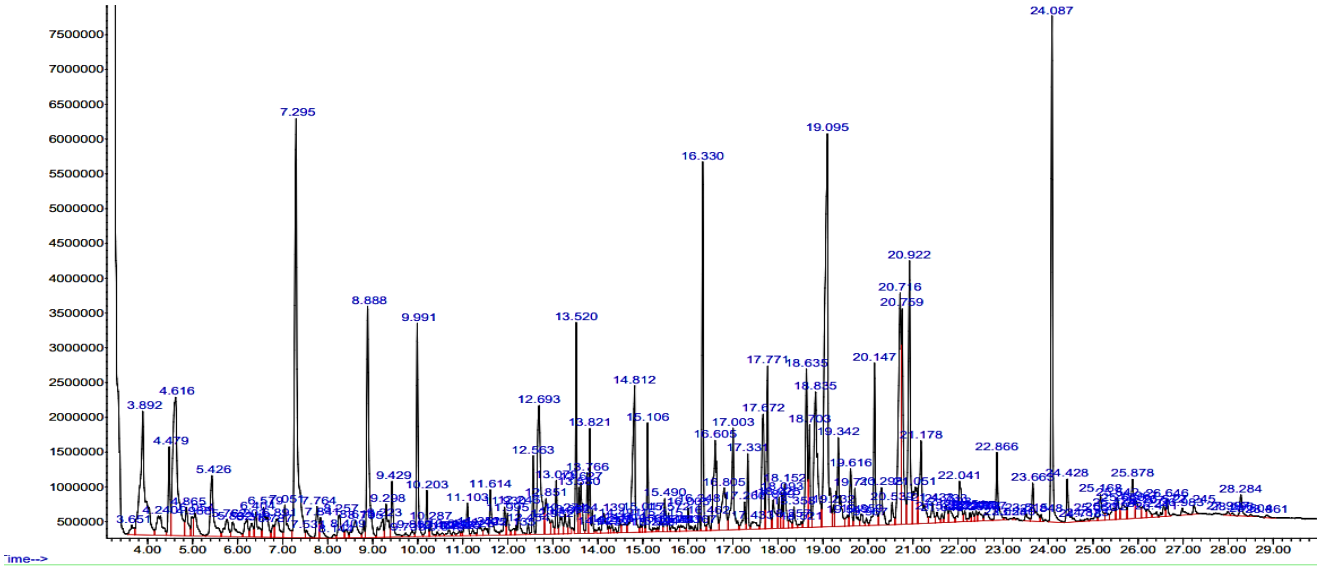


**Figure 3**. GC-MS chromatogram profile of *Bacillus* sp. SA11 ethyl acetate extract

Table 2: Twenty selected compounds from GC-MS profile of ethyl acetate extract with antibacterial activities

| Compound Name | RT (min) | Area (%) | Molecular Formula | Reported Activity | *References* |
|---|---|---|---|---|---|
| n-Hexadecanoic acid | 19,095 | 6.91 | $C_{16}H_{32}O_2$ | Antibacterial | (26) |
| 2-Butoxyethyl acetate | 7,295 | 6.3 | $C_8H_{16}O_3$ | Antibacterial | (27) |
| Bis(2-ethylhexyl) phthalate | 24,087 | 4.45 | $C_{24}H_{38}O_4$ | Antibacterial | (28) |
| Phthalic acid, di(2-propylpentyl) ester | 24,08 | 4.45 | $C_{26}H_{26}O_4$ | Antibacterial | (29) |

| | | | | | |
|---|---|---|---|---|---|
| Benzene, 1,3,5-trimethyl- (Mesitylene) | 4,616 | 3.76 | $C_9H_{12}$ | Antibacterial | (30) |
| Benzaldehyde | 3,892 | 3.19 | $C_7H_6O$ | Antibacterial | (31) |
| 9-Octadecenoic acid (Z)- (Oleic Acid) | 20,716 | 3.17 | $C_{18}H_{34}O_2$ | Antibacterial | (32) |
| Octadecanoic acid (Stearic Acid) | 20,922 | 2.81 | $C_{18}H_{36}O_2$ | Antibacterial | (33) |
| Cyclohexene, 1,6,6-trimethyl- | 8,888 | 2.74 | $C_9H_{16}$ | Antibacterial | (34) |
| 1,2,4-Triazol-4-amine, N-(2-thienylmethyl)- | 16,330 | 2.66 | $C_7H_8N_4S$ | Antibacterial | (35) |
| Pyrrolo (1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- | 18,835 | 2.63 | $C_{11}H_{18}N_2O_2$ | Antibacterial | (36) |
| Benzeneacetamide | 12,693 | 2.37 | $C_8H_9NO$ | Antibacterial | (37) |
| 6-Octadecenoic acid, (Z)- | 20,759 | 2.21 | $C_{18}H_{34}O_2$ | Antibacterial | (38) |
| Dodecanoic acid | 14,812 | 2.11 | $C_{12}H_{24}O_2$ | Antibacterial | (39) |
| 2-Propenoic acid, 2-ethylhexyl ester | 9,991 | 1.78 | $C_{11}H_{20}O_2$ | Antibacterial | (40) |
| Pentadecanoic acid | 17,771 | 1.64 | $C_{15}H_{30}O_2$ | Antibacterial | (41) |
| Diethyltrisulphide | 18,635 | 1.63 | $C_4H_{10}S_3$ | Antibacterial | (42) |
| Tetradecanoic acid (Myristic acid) | 16,605 | 1.51 | $C_{14}H_{28}O_2$ | Antibacterial | (43) |
| 1-Octadecene | 20,147 | 1.43 | $C_{18}H_{36}$ | Antifungal | (44) |
| 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)- | 13,520 | 1.39 | $C_{14}H_{20}O_2$ | Antibacterial | (45) |

## CLINICAL IMPLICATION

This study provides preliminary yet significant clinical implications by demonstrating the broad-spectrum antibacterial activity of *Bacillus* sp. SA11 from mangrove soil against selected Gram-positive (*S. aureus*, *S. mutans*) and Gram-negative (*E. coli*, *K. pneumoniae*) pathogens. In an era of escalating antibiotic resistance, identifying novel

sources of antibacterial compounds is critical. The GC-MS analysis, which pinpointed specific compounds like n-hexadecanoic acid, 2-butoxyethyl acetate, and Phthalic acid, di(2-propylpentyl) ester, provides a crucial starting point for further research. These findings suggest that *Bacillus* sp. SA11 could be a valuable natural resource for isolating and developing new therapeutic agents, potentially contributing to the pipeline of next-generation antibiotics to combat challenging bacterial infections in clinical settings.

## LIMITATIONS

This study provides valuable preliminary findings, but it has several limitations. Primarily, the research utilized a crude ethyl acetate extract of *Bacillus* sp. SA11, meaning the specific active compounds identified by GC-MS were not isolated, purified, or individually tested for their antibacterial efficacy. Consequently, the exact contribution of each identified compound to the observed broad-spectrum activity remains unconfirmed. Furthermore, the study did not quantify the concentration of the active metabolites within the extract, which likely explains the smaller inhibition zones compared to the pure antibiotic positive control. Future research should focus on the isolation, purification, and characterization of these compounds, along with detailed studies on their mechanism of action and in vivo efficacy.

## CONCLUSIONS

This study successfully demonstrated that the ethyl acetate extract of *Bacillus* sp. SA11, isolated from the Ngurah Rai Mangrove Forest, possesses broad-spectrum antibacterial activity against both Gram-positive (*Staphylococcus aureus* and *Streptococcus mutans*) and Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*). Through GC/MS analysis, 165 different compounds were identified, including twenty previously associated with antibacterial properties, with n-hexadecanoic acid, 2-butoxyethyl acetate, Bis(2-ethylhexyl) phthalate, Phthalic acid, di(2-propylpentyl) ester and Benzene, 1,3,5-trimethyl-(Mesitylene) being the most prominent compounds. These results provide strong preliminary evidence that *Bacillus* sp. SA11 from the mangrove ecosystem represents a promising natural resource for the identification and development of new antibacterial compounds.

## CONFLICT OF INTEREST

The authors declare there is no conflict of interest

## AUTHOR CONTRIBUTIONS

NKWDN contributed to the study's conception and design, analysis and interpretation of the data, and drafting of the article. AAGI contributed to the conception and design, provided materials and consumables, and was responsible for data analysis and interpretation, critical intellectual revisions, and final approval. AALP contributed to the study's conception, design, and article drafting, and to data collection and assembly. Lastly, DC provided assistance with data analysis and reviewed the manuscript.

**ACKNOWLEDGMENTS**

The study was financially supported by the Fundamental Research Grant from the Directorate of Research, Technology, and Community Service, Ministry of Education, Culture, Research, and Technology.

**FUNDING**

The study was financially supported by the Fundamental Research Grant from the Directorate of Research, Technology, and Community Service, Ministry of Education, Culture, Research, and Technology for the year 2023, awarded to Anak Agung Gede Indraningrat no grant 3538/LL8/AL.04/2023, 837/UNWAR/LEMLIT/PD-13/2023.

**DECLARATION OF ARTIFICIAL INTELLIGENCE USE**

The authors used AI to assist in improving the language and grammar of the manuscript.

**REFERENCES**

1. Spagnolo AM. Bacterial Infections: Surveillance, Prevention and Control. Pathogens. 2024 Feb 17;13(2):181.
2. Li L, Ma J, Yu Z, Li M, Zhang W, Sun H. Epidemiological Characteristics and Antibiotic Resistance Mechanisms of *Streptococcus pneumoniae*: An Updated Review. Microbiol Res. 2023 Jan;266:127221.
3. Ghajavand H, Kargarpour Kamakoli M, Khanipour S, Pourazar Dizaji S, Masoumi M, Rahimi Jamnani F, et al. Scrutinizing the Drug Resistance Mechanism of Multi- and Extensively-Drug Resistant *Mycobacterium tuberculosis*: Mutations versus Efflux Pumps. Antimicrob Resist Infect Control. 2019 Dec 2;8(1):70.
4. Oliveira M, Antunes W, Mota S, Madureira-Carvalho Á, Dinis-Oliveira RJ, Dias da Silva D. An Overview of the Recent Advances in Antimicrobial Resistance. Microorganisms. 2024 Sep 21;12(9):1920.
5. Gach MW, Lazarus G, Simadibrata DM, Sinto R, Saharman YR, Limato R, et al. Antimicrobial resistance among common bacterial pathogens in Indonesia: a systematic review. The Lancet Regional Health - Southeast Asia. 2024 Jul;26:100414.
6. Muteeb G, Rehman MT, Shahwan M, Aatif M. Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review. Pharmaceuticals (Basel). 2023 Nov 15;16(11):1615.
7. Akram H, Hussain S, Mazumdar P, Chua KO, Butt TE, Harikrishna JA. Mangrove Health: A Review of Functions, Threats, and Challenges Associated with Mangrove Management Practices. Forests. 2023 Aug 23;14(9):1698.
8. Parboteeah S, Jawaheer S, Bhowon MG, Laulloo SJ, Dyall SD. Antibacterial properties and characterization of metabolite extracts of mangrove soil-associated bacteria from a subtropical island: Evaluation of their activity against common human pathogens and biofilms of clinical importance. The Microbe. 2023 Dec;1:100013.
9. Sermkaew N, Atipairin A, Wanganuttara T, Krobthong S, Aonbangkhen C, Yingchutrakul Y, et al. A Novel Bacitracin-like Peptide from Mangrove-Isolated *Bacillus*

*paralicheniformis* NNS4-3 against MRSA and Its Genomic Insights. Antibiotics. 2024 Jul 30;13(8):716.
10. Indraningrat, A. A., Wijaya, M. D., Suryanditha, P. A., Siskayani, A. S., & Janurianti MD. Antibacterial Screening of Bacterial Isolates Associated with Mangrove Soil from the Ngurah Rai Mangrove Forest Bali. Biology Medicine & Natural Product Chemistry. 2021;10(1):129–33.
11. Natania E, Indraningrat AAG, Widhidewi NW. Antibacterial Activity Screening of *Bacillus sp.* AM12 Associated with Mangrove Soil. Biology, Medicine, & Natural Product Chemistry. 2024 Oct 1;13(2):373–9.
12. Indraningrat AAG, Purnami PPCP, Aryastuti AASA, Wijaya MD. Antibacterial Activity of *Pseudomonas aeruginosa* ISP1RL3 Against Multidrug Resistance Bacteria. Jurnal Penelitian Pendidikan IPA. 2023 Dec 20;9(12):11126–36.
13. Indryani V, Chiuman L, Wijaya LL, Lister G, Grandis L. Antibacterial Effect of *Curcuma zedoaria* Extract on *Bacillus cereus* and *Staphylococcus epidermidis*. AMH. 2020;7(1):6–10.
14. Priyanto JA, Prastya ME, Astuti RI, Minart M, Mozef T. Endophytic *Bacillus spp.* isolated from *Archidendron pauciflorum*: Pharmacological property and their phytochemical constituents. Journal of Research in Pharmacy. 2025;27(6):2511–21.
15. Wong KCD, Mongi J, Untu SD, Kanter JW, Rumagit HM, Potalangi NO. Antibacterial Activity Test of Ethanol Extract of *Sonneratia alba* on *Escherichia coli* and *Staphylococcus aureus*. Biofarmasetikal Tropis (The Tropical Journal of Biopharmaceutical). 2025;8(1):44–9.
16. Hong SW, Kim JH, Cha HA, Chung KS, Bae HJ, Park WS, et al. Identification and Characterization of a Bacteriocin from the Newly Isolated *Bacillus sub*tilis HD15 with Inhibitory Effects against *Bacillus cereus*. J Microbiol Biotechnol. 2022 Nov 28;32(11):1462–70.
17. Hernández-González JC, Martínez-Tapia A, Lazcano-Hernández G, García-Pérez BE, Castrejón-Jiménez NS. Bacteriocins from Lactic Acid Bacteria. A Powerful Alternative as Antimicrobials, Probiotics, and Immunomodulators in Veterinary Medicine. Animals (Basel). 2021 Apr 1;11(4).
18. Darbandi A, Asadi A, Mahdizade Ari M, Ohadi E, Talebi M, Halaj Zadeh M, et al. Bacteriocins: Properties and potential use as antimicrobials. J Clin Lab Anal. 2022 Jan;36(1).
19. Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ V. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015 Jan 1;13(1):42–51.
20. Nabi M, Tabassum N, Ganai BA. Phytochemical screening and antibacterial activity of *Skimmia anquetilia* N.P. Taylor and Airy Shaw: A first study from Kashmir Himalaya. Front Plant Sci. 2022;13:937–46.
21. Khedhri S, Polito F, Caputo L, De Feo V, Khamassi M, Kochti O, et al. Chemical Composition, Antibacterial Properties, and Anti-Enzymatic Effects of Eucalyptus Essential Oils Sourced from Tunisia. Molecules. 2023 Oct 21;28(20).
22. Luís C, Algarra M, Câmara J, Perestrelo R. Comprehensive Insight from Phthalates Occurrence: From Health Outcomes to Emerging Analytical Approaches. Toxics. 2021 Jul 1;9(7):157.
23. Baloyi ND, Tekere M, Maphangwa KW, Masindi V. Insights Into the Prevalence and Impacts of Phthalate Esters in Aquatic Ecosystems. Front Environ Sci. 2021 Jul 15;9.

24. Thiemann T. Isolation of Phthalates and Terephthalates from Plant Material – Natural Products or Contaminants? Open Chemistry Journal. 2021 Mar 2;8(1):1–36.
25. Pace A, Vaglica A, Maccotta A, Savoca D. The Origin of Phthalates in Algae: Biosynthesis and Environmental Bioaccumulation. Environments. 2024 Apr 11;11(4):78.
26. Ganesan T, Subban M, Christopher Leslee DB, Kuppannan SB, Seedevi P. Structural Characterization Of N-Hexadecanoic Acid from The Leaves of *Ipomoea eriocarpa* and Its Antioxidant and Antibacterial Activities. Biomass Convers Biorefin. 2024 Jul 30;14(13):14547–58.
27. Leylaie S, Zafari D. Antiproliferative and Antimicrobial Activities of Secondary Metabolites and Phylogenetic Study of Endophytic *Trichoderma* Species From Vinca Plants. Front Microbiol. 2018 Jul 11;9.
28. Javed MR, Salman M, Tariq A, Tawab A, Zahoor MK, Naheed S, et al. The Antibacterial and Larvicidal Potential of Bis-(2-Ethylhexyl) Phthalate from *Lactiplantibacillus plantarum*. Molecules. 2022 Oct 25;27(21).
29. Osuntokun OT, Cristina GM. Bio Isolation, Chemical Purification, Identification, Antimicrobial and Synergistic Efficacy of Extracted Essential Oils from Stem Bark Extract of *Spondias Mombin*(Linn). International Journal of Molecular Biology. 2019;4(4):135–43.
30. Savithri JS, Rajakumar P. Synthesis, Photophysical Property and Antibacterial Activity of Rhodamine B Decorated, Mesitylene Cored and Methylene p-Phenoxy Bridged Dendrimers. ChemistrySelect. 2019 Sep 13;4(34):10143–8.
31. Alonso AM, Reyes-Maldonado OK, Puebla-Pérez AM, Arreola MPG, Velasco-Ramírez SF, Zúñiga-Mayo V, et al. GC/MS Analysis, Antioxidant Activity, and Antimicrobial Effect of *Pelargonium peltatum* (Geraniaceae). Molecules. 2022 May 26;27(11):3436.
32. Veg E, Hashmi K, Ahmad MI, Joshi S, Khan AR, Khan T. Some Biological Applications and Mechanistic Insights of Benzaldehyde-Substituted Thiosemicarbazones and Their Metal Complexes: A Review. Natural Sciences. 2025 Apr 5;5(1–2).
33. Junairiah, Fatimah, Nurhariyat T, Zuraidassa N. Antioxidant, Antimicrobial Activity and Phytochemical Screening of *Syzygium cumini L.* Leaves in Tropical Region from Surabaya, East Java, Indonesia. Asian J Plant Sci. 2023 Jan 15;22(1):104–12.
34. Zhong L, Peng L, Fu J, Zou L, Zhao G, Zhao J. Phytochemical, Antibacterial and Antioxidant Activity Evaluation of *Rhodiola crenulata*. Molecules. 2020 Aug 12;25(16).
35. Strzelecka M, Świątek P. 1,2,4-Triazoles as Important Antibacterial Agents. Pharmaceuticals. 2021 Mar 7;14(3):224.
36. Ridha R, Hefni D, Tachrim ZP, Primahana G, Priyanto JA, Linosefa L, et al. Antibacterial Activity of Endophytic Bacterial Isolates from *Kaempferia galanga* Leaves. Jurnal Kimia Sains dan Aplikasi. 2024 Nov 30;27(11):538–48.
37. Kumar S, Arif M, Kamal M, Jawaid T, Khan MM, Mukhtar B, et al. Ex Vivo Antiplatelet and Thrombolytic Activity of Bioactive Fractions from the New-Fangled Stem Buds of *Ficus religiosa L.* with Simultaneous GC-MS Examination. Molecules. 2023 May 5;28(9).
38. Florenly F, Wijaya CD, Kelvin K, Bao NPG, Dung PCT. Antibacterial Efficacy of Nanoparticles of Rambutan Peel Extracts (*Nephelium lappaceum L.*) compared to Microparticles against Oral Bacteria. e-GiGi. 2022 Mar 13;10(1):95.
39. Vasudevan S, Arulmoorthy MP, Suresh R. Isolation, Purification and Structural Elucidation of Secondary Metabolites from Microcystis *Aeruginosa* Bloom from

Muttukadu Estuary and Its In Vitro Antibacterial, Antioxidant and Anticancer Potency. South African Journal of Botany. 2020 Aug;132:59–67.
40. Shen T, Chen L, Liu Y, Shi S, Liu Z, Cai K, et al. Decanoic acid modification enhances the antibacterial activity of PMAP-23RI-Dec. European Journal of Pharmaceutical Sciences. 2021 Feb;157:105609.
41. Rizvi SNR, Afzal S, Khan K ur R, Aati HY, Rao H, Ghalloo BA, et al. Chemical Characterisation, Antidiabetic, Antibacterial, and In Silico Studies for Different Extracts of *Haloxylon stocksii* (Boiss.) Benth: A Promising Halophyte. Molecules. 2023 May 1;28(9):3847.
42. Wu CY, Wang CY, Sun GJ, Li YQ, Liang Y, Hua DL, et al. Antibacterial characteristics of allyl methyl disulfide and dimethyl trisulfide of *Allium tenuissimum* flower essential oil against Escherichia coli O157:H7. Ind Crops Prod. 2023 Oct;202:117058.
43. Juárez-Rodríguez MM, Cortes-López H, García-Contreras R, González-Pedrajo B, Díaz-Guerrero M, Martínez-Vázquez M, et al. Tetradecanoic Acids With Anti-Virulence Properties Increase the Pathogenicity of *Pseudomonas aeruginosa* in a Murine Cutaneous Infection Model. Front Cell Infect Microbiol. 2020;10:597517.
44. Yap PC, Ayuhan N, Woon JJ, Teh CSJ, Lee VS, Azman AS, et al. Profiling of Potential Antibacterial Compounds of Lactic Acid Bacteria against Extremely Drug Resistant (XDR) *Acinetobacter baumannii*. Molecules. 2021 Mar 19;26(6).
45. Alqahtani MA, Al Othman MR, Mohammed AE. Bio fabrication of silver nanoparticles with antibacterial and cytotoxic abilities using lichens. Sci Rep. 2020 Oct 8;10(1):16781.