Detail Taxonomy of Methycillin Resistant Staphylococcus Aureus and their Biofilm Inhibition

S Srimathi1 , S.Susan1,a)

1Sri Health Centre, Gwalior, Madhya Pradesh, India

**Corresponding Author:** a)[Sairahsusan9@gmail.com](mailto:Sairahsusan9@gmail.com)

**Abstract:** Marine actinobacteria are highlighted as biotechnologically valuable organisms with functional variations, especially in secondary metabolite synthesis, including antibiotics. This study focused on the isolation and identification of a marine actinobacterium identified as *Nocardiopsis sp*., utilizing traditional methods such as sugar utilization patterns, cell wall analysis, and spore chain morphology characterization. The actinobacteria exhibited a long spore chain morphology and showed specific patterns in carbon source digestion and cell wall amino acid analysis, indicating a type III cell wall index.The investigation extended to evaluate the anticancer activity of the marine actinobacteria, particularly focusing on an isoflavonoid compound. The MTT assay on breast cancer cell lines revealed a dose-dependent cytotoxic effect, with apoptosis percentages ranging from 88% at 25μg/ml to 40% at 100μg/ml. Antioxidant activities were assessed using DPPH, H2O2, and Total Antioxidant Activity (TAA) assays. The DPPH assay showed a dose-dependent antioxidant activity for the tested sample, with increasing inhibition percentages at higher concentrations. Hydrogen peroxide scavenging activity and TAA values further confirmed the notable antioxidant potential of the sample compared to the standard antioxidant. Furthermore, the study investigated the biofilm inhibition activity against the pathogen *Staphylococcus aureus*. The marine actinobacteria, at concentrations of 100 and 150 µg/ml, effectively controlled biofilm formation. The results contribute valuable insights into the potential biomedical applications of marine actinobacteria, emphasizing their multifaceted bioactivities and their promising role in combating bacterial infections, particularly in inhibiting biofilm formation by *S. aureus*.

**Keywords:** *Nocardiopsis*; Isoflavonoids; Anticancer; Antioxidant Biofilm; *Staphylococcus aureus*

# Introduction

          Actinomycetes are numerous in natural ecosystems and have the ability to live in a variety of settings. Actinomycetes, also referred to as Gram-positive bacteria, are distinguished by the development of substrate and aerial mycelium on solid substrates as well as the presence of spores with various spore surfaces [(Selim et al., 2021)](https://paperpile.com/c/lJeKZ2/Lx5bm). Marine actinobacteria have a massive biotechnologically valuable organism with functional variation, including variations in secondary metabolite synthesis, particularly antibiotics [(Jagannathan et al., 2021)](https://paperpile.com/c/lJeKZ2/a1Jdg). Actinobacteria, a prolific group of microorganisms renowned for bioactive secondary metabolite production, offer a promising avenue for flavonoid synthesis. Screening programs have uncovered the potential of actinobacteria in producing flavonoids, particularly with notable anticancer activities. [(Marya et al., 2022)](https://paperpile.com/c/lJeKZ2/ROLE2), [(Jain & Verma, 2022; Marya et al., 2022)](https://paperpile.com/c/lJeKZ2/ROLE2+ePnE0), [(Wadhwani et al., 2022)](https://paperpile.com/c/lJeKZ2/06iwo) Given the recognition of marine actinobacteria as sources of novel anticancer agents, they emerge as promising producers of flavonoids with unique structures and properties [(Hozzein et al., 2021)](https://paperpile.com/c/lJeKZ2/AddXe). Isoflavonoids, recognized as natural compounds with broad pharmacological benefits and minimal toxicity, have garnered significant attention in the realms of drug exploration and advancement. The confirmation of metabolic pathways for the biosynthesis of isoflavonoids and flavonoids in microorganisms is well-established. Notably, studies have extensively reported the occurrence of isoflavonoids and flavonoids in fungi and actinomycetes, as highlighted by [(Kang et al., 2016)](https://paperpile.com/c/lJeKZ2/DiTBT). All isoflavonoids and flavonoids derived from actinomycetes are produced by *Streptomyces* because the genes in this genus encode key enzymes such as phenylalanine ammonia-lyase and chalcone synthase (CHS) that catalyze isoflavone and flavone synthesis [(Cui et al., 2019)](https://paperpile.com/c/lJeKZ2/3K1Ka).We delve into the unexplored realm of isoflavonoids sourced from marine actinobacteria, shedding light on their bioactive potential. Our focus extends to understanding the biological activity of these compounds and their efficacy in inhibiting biofilm formation, specifically targeting *Staphylococcus aureus*. *c* secretes an extracellular polymeric substance (EPS), known as biofilm, that helps the microbe to resist and minimise the effect of antibacterial drugs [(Kaplan et al., 2018)](https://paperpile.com/c/lJeKZ2/lrKZp). This exploration aims to contribute valuable insights to the field of microbial research and open avenues for innovative approaches in combating bacterial infections. [(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/lJeKZ2/e0bC7+eQ5Bo+jv8P7), [(Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/lJeKZ2/zWHDY+CHvQ3), [(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/lJeKZ2/QKycx+0fXIv) Bioactive secondary metabolites derived from *Nocardiopsis* encompass a diverse array of compounds, serving as antimicrobial agents, tumor promoters, cytotoxic substances, inhibitors of kinases and P-glycoprotein, immunoregulators, and natural products exhibiting various other bioactivities [(Bennur et al., 2016)](https://paperpile.com/c/lJeKZ2/s80RO). This rich spectrum underscores the potential of *Nocardiopsis* in contributing to a broad range of applications in medicine and beyond.

# Materials and methods

A marine sediment sample was collected from the Rameswaram coast, Tamil Nadu, and carefully transferred into a sterile container. Upon arrival at the laboratory, the sample underwent air-drying for a duration of two weeks. Identification of marine Actinobacteria was carried out by cultivating them on Yeast Malt Agar (YM) medium containing 10g/ml nalidixic acid to inhibit the growth of bacteria and fungi. The sediment samples underwent maceration, followed by successive serial dilution. These diluted samples were then plated on YM agar plates and cultured for one week at room temperature. The population density of Actinobacteria in the sediment samples was quantified in terms of colony-forming units per gram. Colonies with distinct morphologies were identified and selected for pure culture separation and further analysis. The confirmation of marine Actinobacteria identification involved assessing cell wall amino acids, sugar patterns, chemotaxonomic type, and spore chain morphology.The antibiotic resistance profiles of the MRSA isolates were determined using the Kirby-Bauer disk diffusion method, following the CLSI guidelines. Briefly, the bacterial cultures were inoculated onto Mueller-Hinton Agar (MHA) plates, and antibiotic discs containing Amoxiclav (20 µg) and Ertapenem (10 µg) were placed on the agar surface. The plates were incubated at 37°C for 18-24 hours, after which the zones of inhibition were measured. The results were interpreted as susceptible, intermediate, or resistant based on the CLSI breakpoints.The marine actinobacteria *Nocardiopsis sp*. extract was studied for its various antioxidant capabilities, including hydrogen peroxide scavenging activity and 1,1-Diphenyl-2-picryl-hydrazil (DPPH), using the method of [(Kamala et al., 2015; Sivaperumal et al., 2015)](https://paperpile.com/c/lJeKZ2/UoEfR+AKX51) analysis.Additionally, [(Sheefaa & Sivaperumal, 2022)](https://paperpile.com/c/lJeKZ2/knIDP) used the total antioxidant activity method.The six-well plate method was used to evaluate the biofilm's growth. 10 ml of nutrient broth for bacterial pathogens were mixed with a loopful of overnight cultures of the test organisms, and the mixture was then incubated at 37˚C for a full day. Following incubation, the contents of the six well plates were drained out, washed with pH 7.4 phosphate-buffered saline (PBS), and allowed to air dry before being stained with propidium iodide (PI) and 0.1% acridine orange. Any excess staining was removed by washing with distilled water. Subsequently, the six well plates were air-dried upside down and examined for biofilm growth using a confocal microscope.Cytotoxic effects were assessed through MTT activity . Following a 24-hour culture period, cells were subjected to varying concentrations of Nocardia sp. extract for 48 hours. Subsequently, each well received 5 mg/mL of MTT solution, followed by a 4-hour incubation at 37 °C.DMSO-supplemented media served as the control. Negative controls were untreated cells, and positive controls were doxorubicin (10 µg/ml)-treated cells. IC50 values for each cancer cell line were determined using established formulas from prior research. Subsequently, this data was used to plot a linear regression curve for subsequent assays [(Tavares-Carreón et al., 2020)](https://paperpile.com/c/lJeKZ2/WVs4H).

# Results

In the antibiotic susceptibility testing, the MIC values for Amoxiclav and Ertapenem**,** both individually and in combination, were measured for the isolates.The MIC values for Amoxiclav ranged from 4 µg/mL to 256 µg/mL,with most isolates exhibiting resistance (MIC values higher than the clinical breakpoint for Amoxiclav). Specifically, Isolate 1 showed an MIC of 64 µg/mL**,** indicating resistance to Amoxiclav, while Isolate 4 had a very high MIC of 256 µg/mL**,** confirming its resistance.In contrast, the MIC for Ertapenem ranged from 0.25 µg/mL **to** 16 µg/mL**.** The majority of isolateswere susceptible to Ertapenem, with Isolate 5 showing the lowest MIC of 0.25 µg/mL and Isolate 2 being resistant with a MIC of 16 µg/mL (Table 1). However, when Amoxiclav and Ertapenem were combined, the results showed a significant decrease in the MIC values for both drugs, indicating a synergistic effect. For example**,** Isolate 1, which was resistant to Amoxiclav with an MIC of 64 µg/mL, showed a reduced MIC of 8 µg/mL when combined with Ertapenem (MIC of 0.5 µg/mL**).** Similarly, Isolate 4, which was resistant to both Amoxiclav and Ertapenem (MICs of 256 µg/mL and 16 µg/mL, respectively), exhibited a decrease in the MIC values for both drugs when used together, with Amoxiclav reduced to 128 µg/mL and Ertapenem to 8 µg/mL**,** indicating a mild synergistic effect. Overall, these results suggest that combining Amoxiclav and Ertapenem can enhance the antimicrobial efficacy, lowering the MIC of Amoxiclav in resistant strains and potentially improving treatment outcomes for infections caused by *Staphylococcus* sp.

**Table 1.** Antibiotic susceptibility of the pathogen isolated from the oral samples

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Strain** | **Amoxiclav (MIC,µg/mL)** | **Ertapenem (MIC, µg/mL)** | **Amoxiclav + Ertapenem (MIC, µg/mL)** | **Resistance to** | **Susceptible to** |
| 1 | 64 | 2 | 8.5 | Amoxiclav | Ertapenem |
| 2 | 128 | 4 | 17 | Amoxiclav | Ertapenem |
| 3 | 16 | 0.5 | 4.25 |  | Amoxiclav, Ertapenem |
| 4 | 256 | 16 | 136 | Amoxiclav, Ertapenem |  |
| 5 | 8 | 0.25 | 2.25 |  | Amoxiclav, Ertapenem |
| 6 | 32 | 8 | 18 | Amoxiclav, Ertapenem |  |
| 7 | 128 | 16 | 72 | Amoxiclav, Ertapenem |  |
| 8 | 4 | 1 | 2.5 |  | Amoxiclav, Ertapenem |

**Table 2.** Morphological and biochemical characteristics of marine actinobacteria

|  |  |
| --- | --- |
| **Morphological observation** | |
| A.M.Color | White |
| Mel.Pigment | - |
| Sol.Pigment | + |
| Rev.pigment | - |
| Spore | Long chain |
| Cell wall amino-acid | |
| LL-DAP | - |
| DL-DAP | + |
| Glycine | - |
| Alanine | - |
| Lysine | - |
| Ornithine | - |
| Whole cell sugar | |
| Arabinose | - |
| Galactose | ± |
| xylose | - |
| Madurose | - |
| Ribose | ± |
| Sugar pattern | N.C |
| Cellwall type | III |

In the current investigation, traditional methods of identification were employed to identify the marine actinobacterium *Nocardiopsis sp.* The characterization encompassed chemotaxonomic features such as sugar utilization patterns, cell wall analysis, and spore chain morphology. The actinobacteria exhibited a long spore chain morphology. Carbon source digestion revealed positive results for galactose and ribose, while arabinose, xylose, and madurose showed negative utilization. Cell wall amino acid analysis indicated positive results for DL-DAP and negative results for LL-DAP. In terms of cell wall sugar, arabinose, xylose, and madurose exhibited negativity. It was found to be type III cell wall index (Table 2).

DPPH, or 2,2-diphenyl-1-picrylhydrazyl, is a stable free radical commonly used in antioxidant assays to evaluate the antioxidant activity of various compounds. In this study, the antioxidant activities of a sample are measured by assessing their ability to neutralize the DPPH radical.  The DPPH assay indicates a dose-dependent antioxidant activity for the samples tested. Using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, the investigation aimed to measure the antioxidant activity of St. Er and ascorbic acid (Asc. Acid). At various concentrations, the DPPH values (0, 25, 50, 75, and 100μg/ml) were determined. As the concentration of the sample increased, the scavenging activity against DPPH radicals also increased. At a minimum concentration of 25μg/ml, the sample exhibited an inhibition percentage of 20.42%, while the standard antioxidant (St.Er) showed an inhibition of 19.58%. As the concentration further increased to 50, 75, and 100μg/ml, the sample showed increasing inhibition percentages of 38.19%, 54.52%, and 68.34%, respectively. These results suggest that the sample possesses notable antioxidant properties comparable to the standard antioxidant used in the assay. Additionally, the Asc.Acid, which likely represents ascorbic acid, demonstrated consistent and slightly lower inhibition percentages compared to the sample and the standard antioxidant across the concentration range. Further analysis and comparison may be required to fully understand the antioxidant potential of the sample in relation to the reference standards. Hydrogen peroxide (H2O2) is a reactive oxygen species commonly used in biological assays to assess antioxidant activity. The recorded values for the terpenes  at different concentrations of (25,50,75,100) H2O2, along with the  standard error, were compared to the standard ascorbic acid. At 25μg/l H2O2 concentration, the sample exhibited a scavenging activity of 7.43%, with a standard error of 2.4, while the standard ascorbic acid showed a significantly higher scavenging activity of 21.49%, with a standard error of 2.5.As the concentration of H2O2 increased to 50μg/ml, the sample's scavenging activity rose to 19.57%,observed  by a standard error of 2.5. In comparison, the standard ascorbic acid showed a higher scavenging activity of 47.81%, with a standard error of 2.3.At 75μg/ml H2O2 concentration, the sample demonstrated a scavenging activity of 36.04%, with a standard error of 2.7, while the standard ascorbic acid exhibited a higher scavenging activity of 69.75%, with a standard error of 2.5.The maximum H2O2 concentration were at 100μg/ml, resulted in a scavenging activity of 49.42% for the sample, showed by a standard error of 2.6. In comparison, the standard ascorbic acid showed a higher scavenging activity of 78.29%, with a standard error of 2.7. The Total Antioxidant Activity (TAA) assay revealed concentration-dependent antioxidant properties for the sample tested. At a concentration of 25, the sample exhibited a TAA value of 13.07, while the standard antioxidant (St.Er) showed a TAA value of 2.6. As the concentration increased to (50, 75, and 100μg/ml,) the sample demonstrated escalating TAA values of 20.45, 43.92, and 70.95, respectively. The standard antioxidant (St.Er) displayed TAA values of 2.3, 2.7, and 2.5 at the corresponding concentrations. These results suggest that the sample possesses notable total antioxidant activity, surpassing the standard antioxidant across all concentrations. The observed concentration-dependent increase in TAA values indicates a promising antioxidant potential for the sample in comparison to the reference standard used in the assay.The anti-proliferating activity of marine actinobacteria was analyzed by an MTT assay on breast cancer cell lines. Morphological observation and statistical analysis also were done.The cytotoxicity of the isoflavonoid compound was examined  through the MTT assay, observing  the percentage of apoptosis in the treated cells at various concentrations. The results, along with the corresponding standard deviations (St.d), provide insights into the compound's impact on cell viability(Nikalje et al., 2024) (Chehelgerdi et al., 2023). At minimum concentration of 25μg/ml, the compound observed  a cytotoxic effect with a recorded apoptosis percentage of 88%, and a standard deviation of 2.5. As the concentration increased to 50μg/ml, the cytotoxicity persisted, resulting in a reduced apoptosis percentage of 71%, accompanied by a higher standard deviation of 2.7. Further concentration increased to 75μg/ml observed  a continued decrease in cell viability, with the apoptosis percentage decreasing to 59%, and a standard deviation of 2.5. At maximum concentration  at 100μg/ml showed a  cytotoxic effect, as the apoptosis percentage further decreased to 40%, with a standard deviation of 2.4.In this current study, we investigated anti-biofilm activity at various time intervals (0 hours, 24 hours, 48 hours, 72 hours and 96 hours). The effectiveness of Actinobacteria crude extract as a possible medication against *staphylococcus aureus* biofilm activity was examined in a study. When free-floating, planktonic cells adhere to an accessible surface and begin colonizing*, S. aureus* begins to create biofilms. In the study, the control sample exhibited 1011 cells/ml, and at 50 µg/ml, 109 cells/ml were observed. At concentrations of 100, and 150 µg/ml, the cell counts were 108, 106, and 103 cells/ml, respectively.

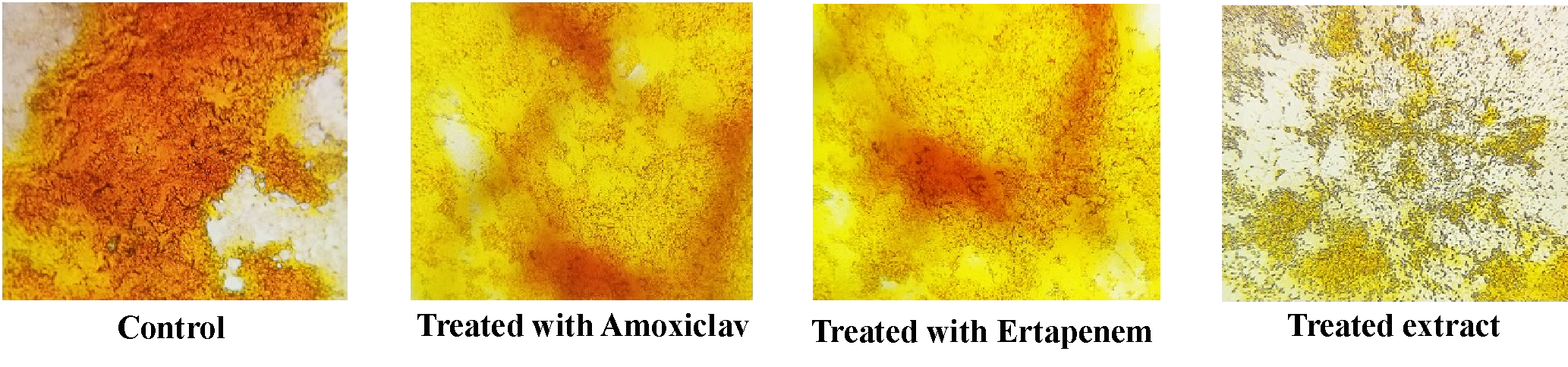


Fig 1:marine antibacteria

# Discussion

395 actinobacterial isolates from marine sediment samples obtained from Persian Gulf sediments have been reported by [(Gozari et al., 2019)](https://paperpile.com/c/lJeKZ2/xKD8q). These isolates show 90% of DPPH radical-scavenging activity in 1250 μg/mL of cell-free extract.Methanol extract is categorized as a potent antioxidant with an IC50 value of 92.17 ppm [(Hidayati et al., 2022)](https://paperpile.com/c/lJeKZ2/VlRiB).[(Vargas-Hernández et al., 2017)](https://paperpile.com/c/lJeKZ2/XZfjT) reported that Capsicum chinense methanolic extracts are increased by hydrogen peroxide foliar discharges; var. Jaguar exhibits the highest activity against both Gram-positive and Gram-negative bacteria, while var. Chichen Itza is most effective against *E. coli* and *E. faecalis*. [(Adel et al., 2023)](https://paperpile.com/c/lJeKZ2/pdxnO), [(Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/lJeKZ2/vBeMW), [(Solanki et al., 2023)](https://paperpile.com/c/lJeKZ2/Akwj5)[(Fahmy & Abdel-Tawab, 2021)](https://paperpile.com/c/lJeKZ2/3pdLi) At concentrations ≤ 31.25 μg/mL, few apoptotic bodies were observed while at concentrations (62.5-500 μg/mL) of the extract all cancer cells exhibited cytoplasm condensation, nuclear margination, and chromatin fragmentation. According to [(Nagaseshu et al., 2016)](https://paperpile.com/c/lJeKZ2/UDhdl), actinobacteria's methanolic extract exhibits possible cytotoxic, antiproliferative, and antioxidant properties. [(Chokkattu et al., 2023)](https://paperpile.com/c/lJeKZ2/wJo1c), [(Laghari et al., 2023; Ramakrishnan et al., 2023)](https://paperpile.com/c/lJeKZ2/4RJxo+kiuI7), [(Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/lJeKZ2/bgVKS) The hydrophobic and hydrophilic interactions that occur between the surface of *S. aureus* cells and any biotic or abiotic material impact the bacteria's ability to attach to a surface [(Maikranz et al., 2020)](https://paperpile.com/c/lJeKZ2/Stoxr). The biofilm formation by Staphylococcus aureus was effectively controlled at concentrations of 100 and 150 µg/ml, as evidenced by confocal microscope images.When isolated from endophytic actinomycetes *Nocardiopsis sp.* GRG 1 (KT235640), Pyrrolo [1,2-a] pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl) shown antibiofilm effectiveness against *E. coli* [*(Rajivgandhi et al., 2018)*](https://paperpile.com/c/lJeKZ2/4z3YJ). [(Dumaru et al., 2019)](https://paperpile.com/c/lJeKZ2/Uiv4a) conducted research to determine the creation of biofilms by Gram-negative bacteria, as well as to determine their antibiograms and detect the production of metallo-beta-lactamases (MBLs) and ESBLs (Extended Spectrum β-Lactamases). [(Muthuswamy Pandian et al., 2022; Ramakrishnan et al., 2023)](https://paperpile.com/c/lJeKZ2/bgVKS+4RJxo), [(Merchant et al., 2022)](https://paperpile.com/c/lJeKZ2/CHvQ3), [(Sreevarun et al., 2023)](https://paperpile.com/c/lJeKZ2/M0wui) Nootkatone, a terpenoid present in grapefruit, demonstrates bacteriostatic and bactericidal effects against *S. aureus*, along with inhibitory properties against biofilm formation, as indicated by [(Farha et al., 2020)](https://paperpile.com/c/lJeKZ2/mvC1o). Essential oil from *Laurus nobilis L*., comprising 1.8-Cineole, methyl eugenol, and α-terpinyl acetate, exhibits robust antibacterial and antibiofilm activities against *S. aureus*, as reported by Merghni in 2015.As anti-biofilm agents, marine bisindole alkaloid 1 and its artificial derivatives have the ability to prevent the formation of microbial biofilms and break down existing ones [(Campana et al., 2019)](https://paperpile.com/c/lJeKZ2/zazpn).

# Conclusion

In conclusion, this study explored the bioactive marine actinobacterial extraction on biofilm inhibition activity against *Staphylococcus aureus*, emphasizing the potential of marine actinobacteria in bacterial infections. These findings contribute valuable insights into the multifaceted bioactivities of marine actinobacteria, particularly *Nocardiopsis*, opening avenues for innovative biomedical applications and drug discovery. The study underscores the importance of exploring natural sources for bioactive compounds with diverse therapeutic potentials.

# References

1. [Adel, S. M., El-Harouni, N., & Vaid, N. R. (2023). White Spot lesions: State of the art biomaterials and workflows used in prevention, progression and treatment. Seminars in Orthodontics. https://doi.org/](http://paperpile.com/b/lJeKZ2/pdxnO)[10.1053/j.sodo.2023.01.002](http://dx.doi.org/10.1053/j.sodo.2023.01.002)
2. [Aparna, J., Maiti, S., & Jessy, P. (2021). Polyether ether ketone - As an alternative biomaterial for Metal Richmond crown-3-dimensional finite element analysis. Journal of Conservative Dentistry : JCD, 24(6), 553–557. https://doi.org/](http://paperpile.com/b/lJeKZ2/e0bC7)[10.4103/jcd.jcd\_638\_20](http://dx.doi.org/10.4103/jcd.jcd_638_20)
3. [Bennur, T., Ravi Kumar, A., Zinjarde, S. S., & Javdekar, V. (2016). Nocardiopsis species: a potential source of bioactive compounds. Journal of Applied Microbiology, 120(1), 1–16. https://doi.org/](http://paperpile.com/b/lJeKZ2/s80RO)[10.1111/jam.12950](http://dx.doi.org/10.1111/jam.12950)
4. [Campana, R., Favi, G., Baffone, W., & Lucarini, S. (2019). Marine alkaloid 2,2-bis(6-bromo-3-indolyl) ethylamine and its synthetic derivatives inhibit microbial biofilms formation and disaggregate developed biofilms. Microorganisms, 7(2), 28. https://doi.org/](http://paperpile.com/b/lJeKZ2/zazpn)[10.3390/microorganisms7020028](http://dx.doi.org/10.3390/microorganisms7020028)
5. [Chokkattu, J. J., Mary, D. J., Shanmugam, R., & Neeharika, S. (2022). Embryonic Toxicology Evaluation of Ginger- and Clove-mediated Titanium Oxide Nanoparticles-based Dental Varnish with Zebrafish. The Journal of Contemporary Dental Practice, 23(11), 1157–1162. https://doi.org/](http://paperpile.com/b/lJeKZ2/QKycx)[10.5005/jp-journals-10024-3436](http://dx.doi.org/10.5005/jp-journals-10024-3436)
6. Chehelgerdi M., Chehelgerdi, M., Allela, O. Q. B., Pecho, R. D. C., Jayasankar, N., Rao, D. P. & Akhavan-Sigari, R. (2023). Progressing nanotechnology to improve targeted cancer treatment: overcoming hurdles in its clinical implementation. Molecular cancer, 22(1), 169.
7. [Chokkattu, J. J., Neeharika, S., & Rameshkrishnan, M. (2023). Applications of Nanomaterials in Dentistry: A Review. Journal of International Society of Preventive & Community Dentistry, 13(1), 32–41. https://doi.org/](http://paperpile.com/b/lJeKZ2/wJo1c)[10.4103/jispcd.JISPCD\_175\_22](http://dx.doi.org/10.4103/jispcd.JISPCD_175_22)
8. [Cui, H., Song, M. C., Ban, Y. H., Jun, S. Y., Kwon, A. S., Lee, J. Y., & Yoon, Y. J. (2019). High-yield production of multiple O-methylated phenylpropanoids by the engineered Escherichia coli-Streptomyces cocultivation system. Microbial Cell Factories, 18(1), 67. https://doi.org/](http://paperpile.com/b/lJeKZ2/3K1Ka)[10.1186/s12934-019-1118-9](http://dx.doi.org/10.1186/s12934-019-1118-9)
9. [Dumaru, R., Baral, R., & Shrestha, L. B. (2019). Study of biofilm formation and antibiotic resistance pattern of gram-negative Bacilli among the clinical isolates at BPKIHS, Dharan. BMC Research Notes, 12(1), 38. https://doi.org/](http://paperpile.com/b/lJeKZ2/Uiv4a)[10.1186/s13104-019-4084-8](http://dx.doi.org/10.1186/s13104-019-4084-8)
10. [Fahmy, N. M., & Abdel-Tawab, A. M. (2021). Isolation and characterization of marine sponge-associated Streptomyces sp. NMF6 strain producing secondary metabolite(s) possessing antimicrobial, antioxidant, anticancer, and antiviral activities. Journal, Genetic Engineering & Biotechnology, 19(1), 102. https://doi.org/](http://paperpile.com/b/lJeKZ2/3pdLi)[10.1186/s43141-021-00203-5](http://dx.doi.org/10.1186/s43141-021-00203-5)
11. [Farha, A. K., Yang, Q.-Q., Kim, G., Zhang, D., Mavumengwana, V., Habimana, O., Li, H.-B., Corke, H., & Gan, R.-Y. (2020). Inhibition of multidrug-resistant foodborne Staphylococcus aureus biofilms by a natural terpenoid (+)-nootkatone and related molecular mechanism. Food Control, 112(107154), 107154. https://doi.org/](http://paperpile.com/b/lJeKZ2/mvC1o)[10.1016/j.foodcont.2020.107154](http://dx.doi.org/10.1016/j.foodcont.2020.107154)
12. [Gozari, M., Bahador, N., Jassbi, A. R., Mortazavi, M. S., Hamzehei, S., & Eftekhar, E. (2019). Isolation, distribution and evaluation of cytotoxic and antioxidant activity of cultivable actinobacteria from the Oman Sea sediments. Hai Yang Xue Bao [Acta Oceanologica Sinica], 38(12), 84–90. https://doi.org/](http://paperpile.com/b/lJeKZ2/xKD8q)[10.1007/s13131-019-1515-2](http://dx.doi.org/10.1007/s13131-019-1515-2)
13. [Hidayati, J. R., Bahry, M. S., Karlina, I., & Yudiati, E. (2022). Antioxidant activity and bioactive compounds of tropical brown algae Padina sp. From Bintan island, Indonesia. Jurnal Kelautan Tropis, 25(3), 309–319. https://doi.org/](http://paperpile.com/b/lJeKZ2/VlRiB)[10.14710/jkt.v25i3.15562](http://dx.doi.org/10.14710/jkt.v25i3.15562)
14. [Hozzein, W. N., Mohany, M., Alhawsawi, S. M. M., Zaky, M. Y., Al-Rejaie, S. S., & Alkhalifah, D. H. M. (2021). Flavonoids from marine-derived Actinobacteria as anticancer drugs. Current Pharmaceutical Design, 27(4), 505–512. https://doi.org/](http://paperpile.com/b/lJeKZ2/AddXe)[10.2174/1381612826666201216160154](http://dx.doi.org/10.2174/1381612826666201216160154)
15. [Jagannathan, S. V., Manemann, E. M., Rowe, S. E., Callender, M. C., & Soto, W. (2021). Marine Actinomycetes, new sources of biotechnological products. Marine Drugs, 19(7), 365. https://doi.org/](http://paperpile.com/b/lJeKZ2/a1Jdg)[10.3390/md19070365](http://dx.doi.org/10.3390/md19070365)
16. [Jain, R. K., & Verma, P. (2022). Visual assessment of extent of White Spot lesions in subjects treated with fixed orthodontic appliances: A retrospective study. World Journal of Dentistry, 13(3), 245–249. https://doi.org/](http://paperpile.com/b/lJeKZ2/ePnE0)[10.5005/jp-journals-10015-2042](http://dx.doi.org/10.5005/jp-journals-10015-2042)
17. [Kamala, K., Sivaperumal, P., Gobalakrishnan, R., Swarnakumar, N. S., & Rajaram, R. (2015). Isolation and characterization of biologically active alkaloids from marine actinobacteria Nocardiopsis sp. NCS1. Biocatalysis and Agricultural Biotechnology, 4(1), 63–69. https://doi.org/](http://paperpile.com/b/lJeKZ2/UoEfR)[10.1016/j.bcab.2014.10.005](http://dx.doi.org/10.1016/j.bcab.2014.10.005)
18. [Kang, H. R., Lee, D., Benndorf, R., Jung, W. H., Beemelmanns, C., Kang, K. S., & Kim, K. H. (2016). Termisoflavones A-C, Isoflavonoid Glycosides from Termite-Associated Streptomyces sp. RB1. Journal of Natural Products, 79(12), 3072–3078. https://doi.org/](http://paperpile.com/b/lJeKZ2/DiTBT)[10.1021/acs.jnatprod.6b00738](http://dx.doi.org/10.1021/acs.jnatprod.6b00738)
19. [Kaplan, J. B., Mlynek, K. D., Hettiarachchi, H., Alamneh, Y. A., Biggemann, L., Zurawski, D. V., Black, C. C., Bane, C. E., Kim, R. K., & Granick, M. S. (2018). Extracellular polymeric substance (EPS)-degrading enzymes reduce staphylococcal surface attachment and biocide resistance on pig skin in vivo. PloS One, 13(10), e0205526. https://doi.org/](http://paperpile.com/b/lJeKZ2/lrKZp)[10.1371/journal.pone.0205526](http://dx.doi.org/10.1371/journal.pone.0205526)
20. [Laghari, I. A., Pandey, A. K., Samykano, M., Aljafari, B., Kadirgama, K., Sharma, K., & Tyagi, V. V. (2023). Thermal energy harvesting of highly conductive graphene-enhanced paraffin phase change material. Journal of Thermal Analysis and Calorimetry, 148(18), 9391–9402. https://doi.org/](http://paperpile.com/b/lJeKZ2/kiuI7)[10.1007/s10973-023-12336-5](http://dx.doi.org/10.1007/s10973-023-12336-5)
21. [Maikranz, E., Spengler, C., Thewes, N., Thewes, A., Nolle, F., Jung, P., Bischoff, M., Santen, L., & Jacobs, K. (2020). Different binding mechanisms of Staphylococcus aureus to hydrophobic and hydrophilic surfaces. Nanoscale, 12(37), 19267–19275. https://doi.org/](http://paperpile.com/b/lJeKZ2/Stoxr)[10.1039/d0nr03134h](http://dx.doi.org/10.1039/d0nr03134h)
22. [Marya, A., Venugopal, A., Karobari, M. I., & Rokaya, D. (2022). White Spot lesions: A serious but often ignored complication of orthodontic treatment. The Open Dentistry Journal, 16(1). https://doi.org/](http://paperpile.com/b/lJeKZ2/ROLE2)[10.2174/18742106-v16-e2202230](http://dx.doi.org/10.2174/18742106-v16-e2202230)
23. [Merchant, A., Ganapathy, D. M., & Maiti, S. (2022). Effectiveness of local and topical anesthesia during gingival retraction. Brazilian Dental Science, 25(1), e2591. https://doi.org/](http://paperpile.com/b/lJeKZ2/CHvQ3)[10.4322/bds.2022.e2591](http://dx.doi.org/10.4322/bds.2022.e2591)
24. [Muthuswamy Pandian, S., Subramanian, A. K., Ravikumar, P. A., & Adel, S. M. (2022). Biomaterial testing in contemporary orthodontics: Scope, protocol and testing apparatus. Seminars in Orthodontics. https://doi.org/](http://paperpile.com/b/lJeKZ2/bgVKS)[10.1053/j.sodo.2022.12.011](http://dx.doi.org/10.1053/j.sodo.2022.12.011)
25. Nikalje, A. V., Tajane, S. T., Kocharekar, A., Vekariya, D., & Patil, H. (2024, April). Detecting Cancer through Analysis of Histopathological Images. In 2024 International Conference on Expert Clouds and Applications (ICOECA) (pp. 579-585). IEEE.
26. [Nagaseshu, P., Gayatridevi, V., Kumar, A. B., Kumari, S., Mohan, M. G., & Malla, R. (2016). Antioxidant and antiproliferative potentials of marine actinomycetes. Int. J. Pharm. Pharm. Sci, 8, 277–284.](http://paperpile.com/b/lJeKZ2/UDhdl) <https://www.academia.edu/download/107951375/admin_Journal_manager_12471_43988_1_CE.pdf>
27. [Pandiyan, I., Sri, S. D., Indiran, M. A., Rathinavelu, P. K., Prabakar, J., & Rajeshkumar, S. (2022). Antioxidant, anti-inflammatory activity of -mediated selenium nanoparticles: An study. Journal of Conservative Dentistry : JCD, 25(3), 241–245. https://doi.org/](http://paperpile.com/b/lJeKZ2/zWHDY)[10.4103/JCD.JCD\_369\_21](http://dx.doi.org/10.4103/JCD.JCD_369_21)
28. [Poornima, P., Krithikadatta, J., Ponraj, R. R., Velmurugan, N., & Kishen, A. (2021). Biofilm formation following chitosan-based varnish or chlorhexidine-fluoride varnish application in patients undergoing fixed orthodontic treatment: a double blinded randomised controlled trial. BMC Oral Health, 21(1), 465. https://doi.org/](http://paperpile.com/b/lJeKZ2/jv8P7)[10.1186/s12903-021-01805-8](http://dx.doi.org/10.1186/s12903-021-01805-8)
29. [Rajivgandhi, G., Vijayan, R., Maruthupandy, M., Vaseeharan, B., & Manoharan, N. (2018). Antibiofilm effect of Nocardiopsis sp. GRG 1 (KT235640) compound against biofilm forming Gram negative bacteria on UTIs. Microbial Pathogenesis, 118, 190–198. https://doi.org/](http://paperpile.com/b/lJeKZ2/4z3YJ)[10.1016/j.micpath.2018.03.011](http://dx.doi.org/10.1016/j.micpath.2018.03.011)
30. [Ramakrishnan, M., Shanmugam, R., Neeharika, S., Selvaraj, S., Chokkattu, J. J., & Thangavelu, L. (2023). Anti-inflammatory potential of a mouthwash formulated using clove and ginger mediated by zinc oxide nanoparticles: An in vitro study. World Journal of Dentistry, 14(5), 394–401. https://doi.org/](http://paperpile.com/b/lJeKZ2/4RJxo)[10.5005/jp-journals-10015-2232](http://dx.doi.org/10.5005/jp-journals-10015-2232)
31. [Ramamurthy, S., Thiagarajan, K., Varghese, S., Kumar, R., Karthick, B. P., Varadarajan, S., & Balaji, T. M. (2022). Assessing the in vitro antioxidant and anti-inflammatory activity of Moringa oleifera crude extract. The Journal of Contemporary Dental Practice, 23(4), 437–442. https://doi.org/](http://paperpile.com/b/lJeKZ2/0fXIv)[10.5005/jp-journals-10024-3323](http://dx.doi.org/10.5005/jp-journals-10024-3323)
32. [Selim, M. S. M., Abdelhamid, S. A., & Mohamed, S. S. (2021). Secondary metabolites and biodiversity of actinomycetes. Journal, Genetic Engineering & Biotechnology, 19(1), 72. https://doi.org/](http://paperpile.com/b/lJeKZ2/Lx5bm)[10.1186/s43141-021-00156-9](http://dx.doi.org/10.1186/s43141-021-00156-9)
33. [Sheefaa, M. I., & Sivaperumal, P. (2022). Antioxidant activities from melanin pigment produced by marine actinobacterium of Streptomyces species. Journal of Advanced Pharmaceutical Technology & Research, 13(Suppl 1), S84–S87. https://doi.org/](http://paperpile.com/b/lJeKZ2/knIDP)[10.4103/japtr.japtr\_338\_22](http://dx.doi.org/10.4103/japtr.japtr_338_22)
34. [Sivaperumal, P., Kamala, K., & Rajaram, R. (2015). Bioactive DOPA melanin isolated and characterised from a marine actinobacterium Streptomyces sp. MVCS6 from Versova coast. Natural Product Research, 29(22), 2117–2121. https://doi.org/](http://paperpile.com/b/lJeKZ2/AKX51)[10.1080/14786419.2014.988712](http://dx.doi.org/10.1080/14786419.2014.988712)
35. [Solanki, L. A., Dinesh, S. P. S., Jain, R. K., & Balasubramaniam, A. (2023). Effects of titanium oxide coating on the antimicrobial properties, surface characteristics, and cytotoxicity of orthodontic brackets - A systematic review and meta analysis of in-vitro studies. Journal of Oral Biology and Craniofacial Research, 13(5), 553–562. https://doi.org/](http://paperpile.com/b/lJeKZ2/Akwj5)[10.1016/j.jobcr.2023.05.014](http://dx.doi.org/10.1016/j.jobcr.2023.05.014)
36. [Sreevarun, M., Ajay, R., Suganya, G., Rakshagan, V., Bhanuchander, V., & Suma, K. (2023). Formulation, Configuration, and Physical Properties of Dental Composite Resin Containing a Novel 2π + 2π Photodimerized Crosslinker - Cinnamyl Methacrylate: An Research. The Journal of Contemporary Dental Practice, 24(6), 364–371. https://doi.org/](http://paperpile.com/b/lJeKZ2/M0wui)[10.5005/jp-journals-10024-3480](http://dx.doi.org/10.5005/jp-journals-10024-3480)
37. [Subramanian, A., & Harikrishnan, S. (2023). 3D printing in orthodontics: A narrative review. Journal of International Oral Health: JIOH, 15(1), 15. https://doi.org/](http://paperpile.com/b/lJeKZ2/vBeMW)[10.4103/jioh.jioh\_83\_22](http://dx.doi.org/10.4103/jioh.jioh_83_22)
38. [Tavares-Carreón, F., De la Torre-Zavala, S., Arocha-Garza, H. F., Souza, V., Galán-Wong, L. J., & Avilés-Arnaut, H. (2020). In vitro anticancer activity of methanolic extract of Granulocystopsis sp., a microalgae from an oligotrophic oasis in the Chihuahuan desert. PeerJ, 8(e8686), e8686. https://doi.org/](http://paperpile.com/b/lJeKZ2/WVs4H)[10.7717/peerj.8686](http://dx.doi.org/10.7717/peerj.8686)
39. [Vargas-Hernández, M., Torres-Pacheco, I., Gautier, F., Álvarez-Mayorga, B., Cruz-Hernández, A., García-Mier, L., Jiménez-García, S. N., Ocampo-Velázquez, R. V., Feregrino-Perez, A. A., & Guevara-Gonzalez, R. G. (2017). Influence of hydrogen peroxide foliar applications onin vitroantimicrobial activity inCapsicum chinenseJacq. Plant Biosystems, 151(2), 269–275. https://doi.org/](http://paperpile.com/b/lJeKZ2/XZfjT)[10.1080/11263504.2016.1168494](http://dx.doi.org/10.1080/11263504.2016.1168494)
40. [Verma, P., & Muthuswamy Pandian, S. (2021). Bionic effects of nano hydroxyapatite dentifrice on demineralised surface of enamel post orthodontic debonding: in-vivo split mouth study. Progress in Orthodontics, 22(1), 39. https://doi.org/](http://paperpile.com/b/lJeKZ2/eQ5Bo)[10.1186/s40510-021-00381-5](http://dx.doi.org/10.1186/s40510-021-00381-5)
41. [Wadhwani, V., Sivaswamy, V., & Rajaraman, V. (2022). Surface roughness and marginal adaptation of stereolithography versus digital light processing three-dimensional printed resins: An study. Journal of Indian Prosthodontic Society, 22(4), 377–381. https://doi.org/](http://paperpile.com/b/lJeKZ2/06iwo)[10.4103/jips.jips\_8\_22](http://dx.doi.org/10.4103/jips.jips_8_22)