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RESEARCH ARTICLE OPEN ACCESS

Marine-Derived *Bacillus subtilis* as a Source of Bioactive Coumarin Derivative-Isolation and Characterization

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Abstract

Marine microorganisms are gradually recognized as a prolific source of bioactive compounds with promising applications in therapeutic research. In this study, a marine bacteria *Bacillus subtilis* was isolated and identified using 16S rRNA sequencing for the production of bioactive compound Coumarin derivative. The compound Coumarin derivative (2H-1-Benzopyran-2-one, 3-amino-4-hydroxy), was extracted using solvent extraction, column purification method and identified through UV Spectrometry, FTIR and GCMS analysis, the Spectrometric characterization confirmed the presence of amino and hydroxyl substitutions on the Coumarin core, enhancing the compound's biological profile. It exhibited significant antimicrobial action against test microorganisms and cytotoxic activity against (MDA-MB-231) human breast cancer cell line. These findings highlight the potential of marine *Bacillus subtilis* derived Coumarin as lead molecules for developing dual-purpose antimicrobial and anticancer therapeutics.

Keywords: Bioactive Compound, Coumarin Derivative, Antimicrobial Activity, Cytotoxic Activity, *Bacillus subtilis*

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INTRODUCTION

The growing threat of antimicrobial resistance and the persistent worldwide burden of cancer point out the critical necessity for novel bioactive compounds with dual therapeutic potential.[1] Marine ecosystems, particularly marine-derived microorganisms, have emerged as prolific sources of structurally unique and pharmacologically potent secondary metabolites.[2,3] Marine *Bacillus* species are well-known producers of antimicrobial peptides. *Bacillus amyloliquefaciens* produces bacillomycin, a potent antifungal agent.[4] The probiotic *Bacillus clausii* strain produces a sensitive antimicrobial substance known as pronase during the stationary growth phase. Among these, *Bacillus* species are recognized for their remarkable biosynthetic capabilities, especially in producing heterocyclic compounds with diverse biological functions. *Bacillus subtilis* synthesizes several antimicrobial compounds, including bacitracin, subtilin, and fengycin.[5] Bacitracin a polypeptide antibiotic is effective against Gram-positive bacteria. The isolation and characterization of this peptide revealed as effective against wide range of organisms, suggesting therapeutic application in pharmaceutical research and therapeutic formulations.[6]

Coumarins, a class of benzopyrone compounds, are well-known for their broad-spectrum pharmacological activities, including anticancer, microbicidal, anti-inflammatory and anti-oxidant properties.[7] These compounds are characterized by a fused benzene and α-pyrone ring, and natural as well as semi-synthetic derivatives have demonstrated significant therapeutic relevance.[8] The marine-derived coumarins are of special interest due to their enhanced bioactivity, likely influenced by the extreme and competitive environmental conditions of the ocean that drive the evolution of unique metabolic pathways in marine microbes.[9,10] Recent studies have reported the isolation and characterization of a Coumarin derivative from *Bacillus* sp. isolated from marine habitats, exhibiting potent antimicrobial activity against pathogenic bacteria as well as notable cytotoxic effects against human cancer cell lines.

In this study, we report the isolation and characterization of a hydroxylated amino-coumarin derivative, synthesized by a marine *Bacillus subtilis* and its graphical representation is shown in Figure 1. This compound features

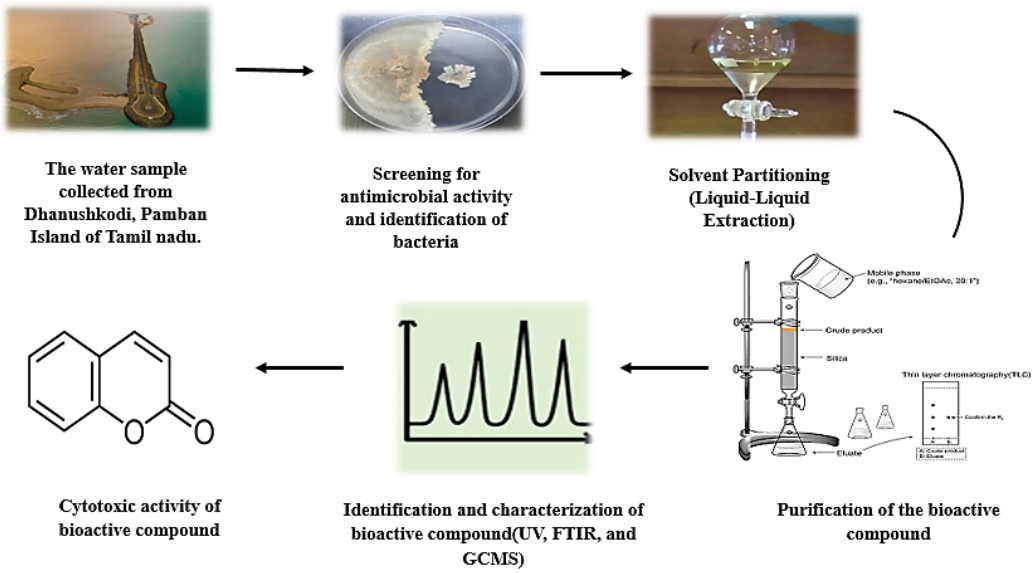

Figure 1. Graphical representation of isolation and characterization of a hydroxylated amino-coumarin derivative, synthesized by a marine *Bacillus subtilis*

both hydroxyl and amino substitutions on the coumarin core, structural modifications that are associated with enhanced solubility, redox potential, and biological activity. The 3-amino and 4-hydroxy groups are particularly notable for their role in modulating antimicrobial efficacy, potentially by interacting with bacterial DNA gyrase, topoisomerase, or membrane-associated proteins, leading to disruption of microbial growth.

Additionally, the anticancer activity of this derivative may be attributed to its capacity to interfere with cancer cell proliferation, possibly through the generation of reactive oxygen species, induction of mitochondrial dysfunction, or modulation of apoptosis-related pathways. The presence of both antimicrobial and cytotoxic properties in a single molecule highlights the therapeutic promise of marine microbial metabolites, especially for use in combination therapies or as lead compounds in multi-target drug discovery.

MATERIALS AND METHODS

Isolation of marine bacteria

The marine bacteria *Bacillus* sp. (MO5) was isolated from the water sample collected from Dhanushkodi, Pamban Island of Tamil Nadu. The sample was serially diluted using the method serial dilution agar plating was followed to isolate bacteria from sample using HiMedia ZMA (Marine Agar 2216) at 20-35 °C for 2-5 days.[11] Colonies developed after incubation was purified by repeated sub culturing on marine ZMA media, in which the bacterial isolates was streaked quadrant as shown in Figure 2 to get a pure culture based on their identical colony morphology such as elevation, margin opacity, colour and surface appearance.[12,13]

Identification of bacterial strain

The cultural characteristics of the bacterial strain MO5 was studied with the growth of colonies on the media, characteristics like pigment production, shape of the colonies, and formation of spore were recorded under microscope (Table 1).[14] Gram staining method (Gram 1884, and O'Toole, 2016) was done on pure marine isolates obtained results as in Figure 3.[15,16]

Biochemical Characterization and molecular

HiMedia ready to use biochemical kit KB013 was used for biochemical characterization of the marine bacteria (MO5). This kit works on the principle of colorimetric test system, standardized, based on carbohydrate utilization and other biochemical tests. All the tests number in the Figure 4 listed in the Table 2 as sterile media used for biochemical testes to identify the Marine isolate, after incubation the colour of media gets changed due to the bacteria exhibits some metabolic changes which can be either interpreted visually or after addition of reagents where ever it is required such as Baritt reagent A (R029) and B (R030) for VP test, Sulphanilic Acid 0.8% (R015) for Nitrate test, TDA Reagent (R036) for Phenylalanine deamination, and α-Naphthylamine Solution (R009) for Nitrate test.

About 50 µL or a loopful of inoculum was placed on each well of the HiMedia Kit and incubated at 35 °C for 24 hours. After the incubation a series of reagents was added to respective wells and biochemical test results were interpreted based on the colour change.[17]

The 16S rRNA sequence analysis was performed to determine the species at molecular level and phylogenetic tree was constructed to map the interrelationship of the species with other organisms. For this the PCR amplification and DNA sequencing of 16S rRNA gene from genomic DNA was carried out.

Solvent extraction method

The inoculum of the isolate MO5 (*Bacillus subtilis*) was prepared by adding 5 mL of 3 days old culture aseptically into Erlenmeyer flasks containing 250 mL of optimized medium (the Zobells marine broth 2216 HiMedia, with 3% Nacl and pH 7.0) along with control. After inoculation, the flask was incubated in an orbital shaker with 150 rpm at 35 °C for 48 hrs and the seed culture was used for the huge production of antimicrobial secondary metabolites.[18] Control fermentations using sterile Zobell marine broth showed no detectable coumarin-like compounds, confirming that the growth medium was not the source of the molecule. The compound was reproducibly detected only in inoculated cultures of *Bacillus subtilis*.

Culture purity was ensured through repeated streaking, Gram staining, biochemical characterisation, and 16S rRNA sequencing. Extraction blanks further ruled out laboratory contamination. The large scale production fermentation was carried out with 10% of culture broth, inoculated into 1 L flaks of optimized production medium at 35 °C for 3 days. The fermented broth collected at the end of 72 hrs was filtered with Whatman No. 42 (Merck, Mumbai, India), The filtrate obtained after filtration was extracted twice with an equal volume of ($C_4H_8O_2$) ethyl acetate (Sigma- Aldrich, CAS Number: 141-78-6), combined and the organic layer was evaporated to dryness in a rota evaporator, the crude extract was collected aseptically and used for further characterization.

Purification of the bioactive compound

Purification of crude extract was carried out by column chromatography using silica gel column. Separation is done using the gradient elution technique. Silica gel (100-200 mesh) slurry without air bubble was placed in the column as stationary phase. The solvent system Ethyl acetate (Sigma-Aldrich, CAS Number: 141-78-6) and chloroform (CAS 67-66-3) in (8:2) was used as mobile phase. 10 mg of the extract was dissolved in 1 mL of 1% DMSO and carefully loaded onto the column. Fractions or eluents with retention factors (RF) was combined and checked for antimicrobial activity against the test pathogens *Escherichia coli*, *Aspergillus niger*, and *Candida albicans* using agar diffusion bioautography method.[19]

To further confirm antibacterial activity and associate it directly with the isolated compound, an agar bioautography (autobiography) assay was performed. After TLC separation, the developed plate was overlaid with agar seeded with test bacteria. A clear inhibition zone appeared precisely at the Rf position corresponding to the isolated coumarin derivative. This confirms that the purified band contains the active antibacterial molecule and directly links antibiosis to the identified compound.

Identification and characterization of bioactive compound

The fraction of MO5 (*Bacillus subtilis*) with high antimicrobial activity were characterized with the analytical techniques like UV spectrometry, FTIR, and GC-MS.

UV spectrometry

UV spectrometry is the most common analytical study used for quantitative identification of high conjugated organic compounds, and biological macromolecules.[20] The purified compound MO5 (*Bacillus subtilis*) was dissolved in ethyl acetate and subjected to UV spectral analysis by using UV-1800 [SHIMADZU], the optical density for absorbance measurement was carried from 200-800 nm.

FTIR analysis

The FTIR analysis was carried out with the Bruker ALPHA-T FTIR Spectrophotometer which is a compact and reliable instrument for infrared spectroscopy, ideal for identifying functional groups and molecular structures. The KBr pellet method was used for analysis of unknown antimicrobial compound samples in FTIR spectroscopy by embedding them in infrared-transparent KBr. 2 mg of dried unknown compound was grinded with 100 mg of dry KBr powder. The mixture was pressed in a hydraulic press (~10 tons, 1-2 min) to create a transparent pellet. The pellet was placed in the holder, and background scan was performed, then spectrum was scanned from the range of 4000-400 cm^{-1} range using OPUS software. The spectrum was analysed to identify the functional groups present in the compound.[21]

Gas Chromatography-Mass Spectrometry

The GC-MS analysis was performed to identify the unknown compound. 1 µL of compound sample was injected into silica column (30 m × 0.25 mm ID × 250 µm df) an Elite-5ms fused with helium as the carrier gas flow 1 mL/min at 60 °C for 2 min and gradually increased with interval of 10 °C/min to 300 °C and held for 6 min. The mass spectrometer was functioned with electron impact of (70 eV) mode, with a transfer line and ion source temperature of 240 °C. Spectra were recorded in the 40-600 Da range and matched with the GC-MS NIST-2008 library for compound identification.[22,23]

***In vitro* cytotoxic activity (MTT assay)**

Cytotoxic activity (MTT assay) of the purified compound Coumarin derivative was assessed on Triple-negative breast cancer (TNBC) cell line MDA-MB-231 which was procured from National centre for cell science.[24,25] 10000 cells per well was maintained as culture in 96 well plate in Dulbecco's Modified Eagle Medium (AT149-IL) supplemented with 10% (v/v) fetal bovine serum (HIMEDIA-RM 10432) and 1% Penicillin-Streptomycin antibiotic solution (Sigma-Aldrich P0781) at 37 °C with 5% CO_2.[26,27]

Next day cells were treated from different concentrations such as 10, 20, 40, 60, 80 and 100 µg of sample as out lined by Abu-Farich et al.[28,29] After 24 hours of incubation, MTT solution was added to cell culture and further incubated (Air-jacketed CO_2 incubator-heal force-HF90) for 2 hours, culture supernatant was removed and cell layer matrix was dissolved in 100 µl DMSO and the absorbance of the MTT formazan was read at 540 nm in an Elisa plate reader (iMark, Biorad, USA) along with control and blank.

The statistical data of the IC_{50} was calculated by using software Graph Pad Prism -9, two way ANOVA Tukey's post hoc test. 50% inhibitory concentration (IC_{50}) significance value was set at $P < 0.001$.

RESULTS AND DISCUSSION

Isolation of marine bacteria

The cultural, morphological, physiological, and biochemical characteristics of the marine bacterial isolates MO5 was analysed, along with genomic data, to facilitate their identification through a polyphasic taxonomic approach. The bacterial isolate was cultivated on Zobell marine media, and their colony morphology was observed as illustrated in Figure 2. It shows that Colonies were large, irregular, and either flat or slightly raised with a rough texture. They were off-white in colour and produced a mild, earthy smell.

Identification of bacterial strain

A morphological study was conducted to identify the marine bacteria. The results of this analysis, including staining characteristics, are presented in Table 1. Gram staining was performed, and the stained samples were microscopically examined under 10X, 40X, and oil immersion objectives to observe cell morphology.[30] the shape and staining properties of the bacteria was depicted in Figure 3.

Table 1. The results of morphological characteristics of MO5

No.	Morphological characteristics	Results of MO5 bacterial strain identification
1	Cell Shape	Rod-shaped
2	Gram Staining	Gram-positive
3	Motility	Motile
4	Spore Formation	Spore-forming

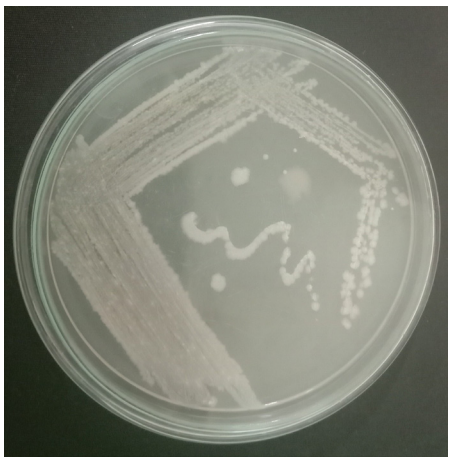

Figure 2. Potent marine isolates MO5 on Zobell marine agar

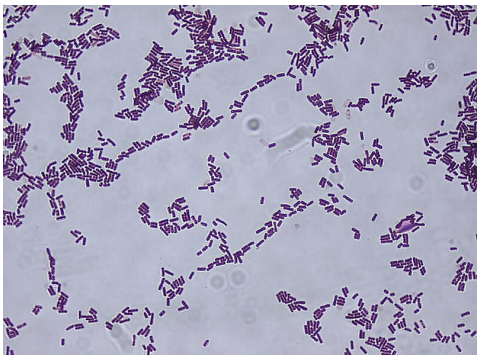

Figure 3. Gram staining of marine bacteria MO5

Biochemical Characterization

The biochemical characterization of isolate MO5 was shown in Figure 4, with the corresponding biochemical test results provided (Table 2). Based on the interpretation results using the HiMedia biochemical kit KB013, the bacterial identification index indicates that the microorganism belongs to the *Bacillus* sp.

To confirm the identity of bacteria strain MO5 at the molecular level, 16S rRNA gene sequencing was performed. This method also facilitated the construction of a phylogenetic tree to elucidate the evolutionary relationship of potent isolates with other closely related microorganisms. The Basic Local Alignment Search Tool (BLAST) to identify homologous sequences from the National Center for Biotechnology Information (NCBI) database. BLAST compares nucleotide or protein sequences against database entries to identify regions of local similarity and calculates the statistical significance of the matches.

Strain MO5 was analysed using the UPGMA method with the JTT matrix-based model, including 1000 bootstrap replicates. Only branches with greater than 50% bootstrap support was retained. The analysis used 10 amino acid

Table 2. Biochemical test results interpretation of marine bacteria MO5

No.	Biochemical test	Results interpretation of Isolate MO5
1	Malonate	Negative
2	Voges Proskauer's	Positive
3	Citrate	Positive
4	ONPG	No change
5	Nitrate reduction	Positive
6	Catalase	After incubation, on addition of 3% H_2O_2 the Effervescence was observed
7	Arginine utilization	No change
8	Sucrose	Weak Positive
9	Mannitol	Weak Positive
10	Glucose	Positive
11	Arabinose	No change
12	Trehalose	Positive

Figure 4. Biochemical characterization of marine isolate MO5

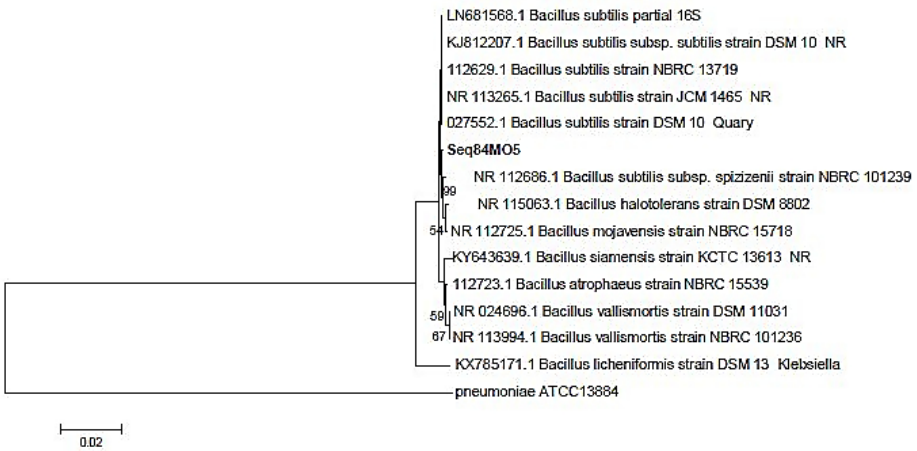

Figure 5. Phylogenetic tree of MO5 showed close homology with the strain CP019663 *Bacillus subtilis*

sequences, with gaps and missing data removed, producing a final dataset of 784 positions. Phylogenetic analysis result is presented in Figure 5.

The biochemical, morphological, and molecular data show strong match with reference to *Bacillus subtilis* strains. The isolate displayed Gram-positive rod morphology, endospore formation, catalase positivity, and starch/casein hydrolysis, all characteristic of *B. subtilis*. 16S rRNA sequencing showed >99% similarity to *B. subtilis* strains deposited in NCBI and matched ATCC reference sequences. Phylogenetic analysis placed the isolate firmly within the *B. subtilis* clade with strong bootstrap support. These results confirm reliable identification and taxonomic placement.

Genomic nucleotide sequences was submitted to the GenBank database using the BANKIT tool. Accession number ON032864 was obtained for the Marine isolate MO5.[31]

Table 3. Column chromatography results of MO5 (*Bacillus subtilis*)

Preparation of column chromatography	Results and materials used for the purification of the compound
Stationary phase	Silica gel (100-200 mesh)
Mobile phase	Chloroform:Ethyl acetate (8:2)
Solvent required for the column	500 ml
Drops collected per minute	8-10 drops
Each eluate volume is approximately	65 ml
Number of fractions collected	7

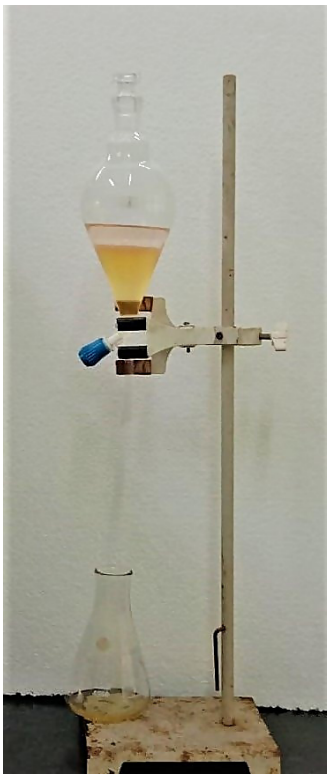

Figure 6. Solvent extraction or Solvent Partitioning (Liquid-Liquid Extraction), process using MO5 (*Bacillus subtilis*) marine bacterial filtrate and ethyl acetate (1:1)

Solvent extraction method

Solvent partitioning (liquid-liquid extraction) was performed on the fermented broth collected at the end of the incubation period for MO5 (*Bacillus subtilis*) at 48 hours, the broth was filtrated with Whatman No. 42 filter paper

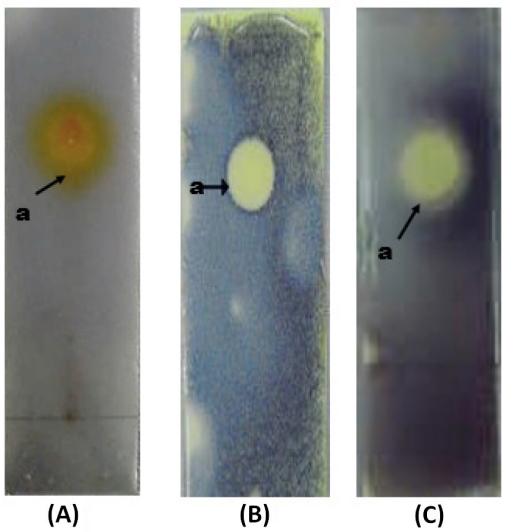

Figure 7. Bio-autography of compound from MO5 (*Bacillus subtilis*) with test pathogens (A) *E. coli;* (B) *Aspergillus niger* and (C) *Candida albicans*

(Merck, Mumbai, India).[32] The resulting culture filtrate was extracted with an equal volume of ethyl acetate, and the combined organic phase (1:1) was evaporated to dryness using a rotary evaporator, as shown in Figure 6. The resulting crude extract was collected aseptically and used for further characterization.

Purification of the bioactive compound

Column chromatography separates compounds based on their differential adsorption to the stationary phase, allowing them to migrate through the column at varying rates and thus enabling their separation into distinct fractions. The preparation and results of column chromatography are summarized in Table 3.

Table 4. FTIR Peaks assigned for Coumarin derivative, 2H-1-Benzopyran-2-one, 3-amino-4-hydroxy

No.	Wavenumber (cm^{-1})	Vibration type	Functional group assignment
1	3312	N-H Stretch	Primary or secondary amine (-NH)
2	3221	O-H Stretch (broad)	Phenol or alcohol (-OH)
3	1725	C=O Stretch	Lactone ring or ester carbonyl group
4	1526	C=C Stretch (aromatic ring)	Aromatic system (coumarin ring)
5	1265	C-N Stretch	Amine or amide group

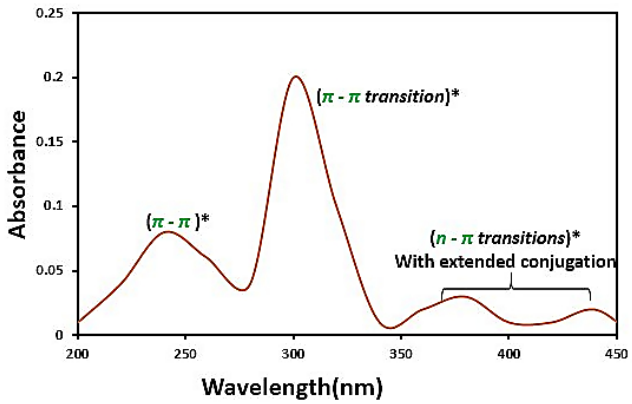

Figure 8. UV-Vis spectrum for compound MO5 (*Bacillus subtilis*)

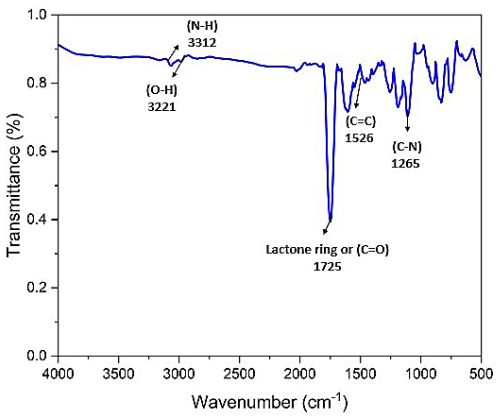

Figure 9. FTIR spectrum of MO5 (*Bacillus subtilis*) compound (Coumarin derivative)

All compounds collected from the chromatographic fractions were evaluated for antimicrobial activity using the agar diffusion bioautography method, a simple and direct approach for detecting bioactive compounds. Among the tested fractions, compound from

Table 5. List of potent compounds from MO5 (*Bacillus subtilis*) marine bacteria

Peak	R. Time	Name of the compound
1	1.319	4-chlorobuten-3-yne
2	13.939	2H-1-benzopyran-2-one, 3-amino-4-hydroxy
3	19.831	Cyclohexanone, 2, 2-Dimethyl

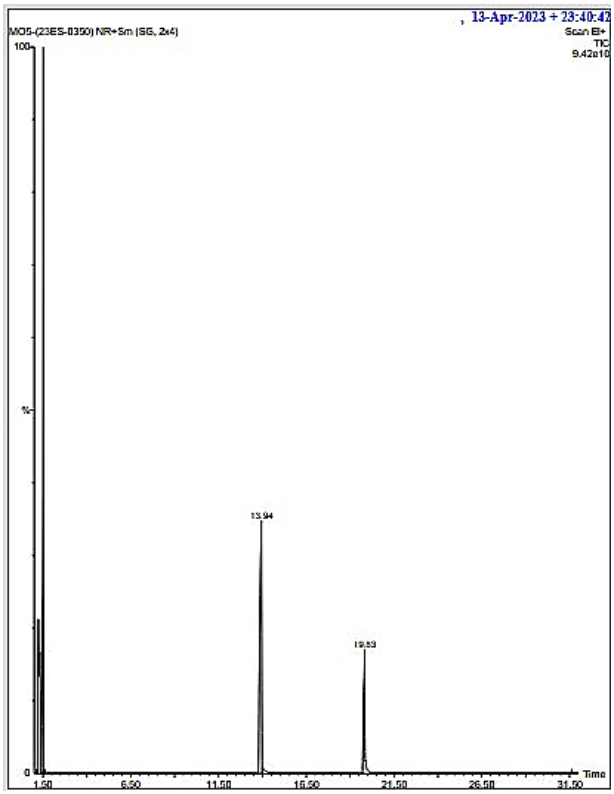

Figure 10. The GC-MS Spectral image of MO5 (*Bacillus subtilis*)

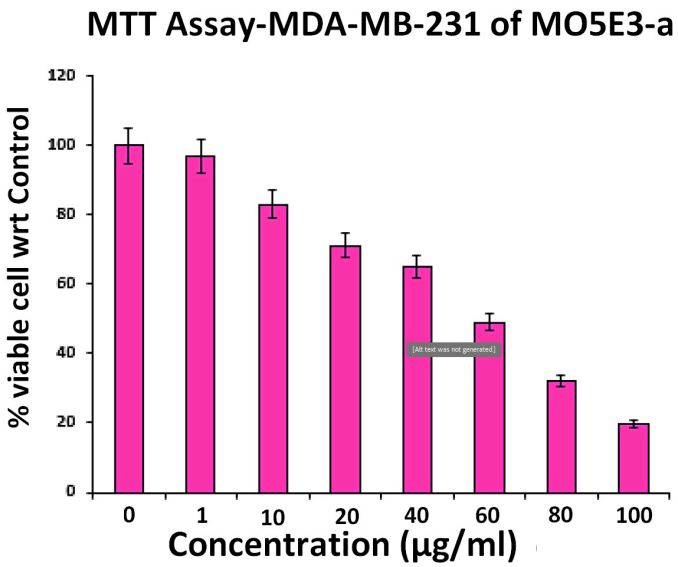

Figure 11. Graphical representation of cytotoxic activity of purified compound MO5E3-a (2H-1-Benzopyran-2-one, 4-hydroxy). Values are expressed as Mean ± SD, Statistical significance (P ≤ 0.05)

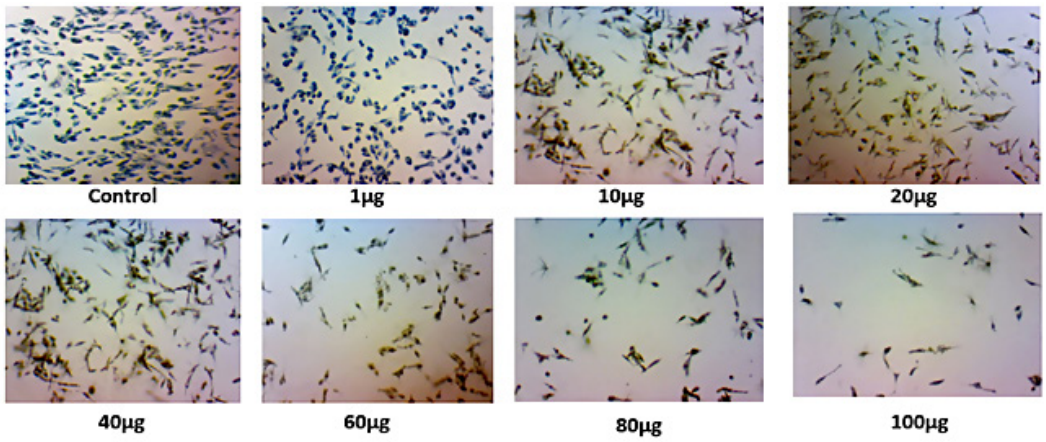

Figure 12. *In vitro* cytotoxic activity of purified compounds with MTT assay on cell line MDA-MB-231

Fraction 3 of the marine bacterium MO5 (*Bacillus subtilis*) exhibited broad-spectrum antimicrobial activity. This compound was effective against all test organisms, including both bacterial and fungal strains *Escherichia coli, Aspergillus niger*, and *Candida albicans* as shown in Figure 7.

Identification and characterization of bioactive compound

The UV-Visible absorbance spectrum of compound (MO5E3-a) was recorded over a wavelength range of 200-500 nm. As shown in Figure 8, the major absorption peak was observed near 300 nm, indicating the presence of a conjugated system with a strong chromophore. This peak corresponds to $\pi \rightarrow \pi^*$ electronic transitions, which are characteristic of the coumarin scaffold.[33] The presence of amino ($-NH_2$) and hydroxyl (-OH) groups likely causes a slight bathochromic shift (redshift) due to increased conjugation and possible intramolecular hydrogen bonding. The prominent peak at ~300 nm confirms the presence of lactone and amine functional groups.

A secondary peak in the 230-250 nm region corresponds to $\pi \rightarrow \pi^*$ transitions within the benzopyran ring, with additional contributions from electron-donating groups such as hydroxyl and amino functionalities. The intensity of this peak reflects strong electronic delocalization within the coumarin system. Minor absorbance features observed between 350 and 450 nm may be attributed to $\pi \rightarrow \pi^*$ transitions involving lone pairs on oxygen and nitrogen atoms. The hydroxyl and amino groups may also facilitate slight charge transfer interactions, which extend the absorption tail in this region.

Strict aseptic techniques were used throughout isolation and fermentation. The strain was purified through multiple streaking cycles and confirmed as *Bacillus subtilis* by morphology, biochemistry, and 16S rRNA sequencing. Sterile medium controls and solvent blanks showed no metabolic peaks, confirming that the bioactive molecule originated solely from the purified isolate.

FTIR analysis

The FTIR spectrum of compound Coumarin derivative as presented in Figure 9 revealed several characteristic absorption bands indicative of key functional groups.[34] A major peaks observed in the 4000-500 cm^{-1} range is mentioned in Table 4.

Gas chromatography-mass spectrometry

The solubility of the purified compound from MO5 (*Bacillus subtilis*) was assessed in various solvents, including ethyl acetate, ethanol, methanol, and DMSO. The GC-MS spectral profile of the compound is presented in Figure 10. Table 5 shows the presence of 2H-1-benzopyran-

2-one, 3-amino-4-hydroxy with the retention time 13.939.34.

UV, FTIR, and GC-MS analyses provided a consistent structural profile corresponding to a coumarin derivative. Although NMR spectra could not be recorded due to limited sample availability, the combined spectral data closely matched reference coumarin derivatives reported in the previous literature. Future work will include full NMR characterization using larger quantities of purified compound.

Cytotoxic activity of bioactive compound

The cytotoxic activity of the purified compounds MO5E3-a (2H-1-Benzopyran-2-one, 4-hydroxy) was evaluated using the MTT assay on the MDA-MB-231 cell line. Treatment of MDA-MB-231 cells with the compound was resulted in concentration-dependent inhibition of cell growth, as shown in Figure 11.

The IC_{50} value, defined as the concentration of the compound at which cell viability is reduced by 50%, was calculated to assess the potency of each compound. Lower IC_{50} values indicate higher cytotoxic potency. The percentage of viable cells was determined using the following formula:

$$\% \text{ Viable cells} = (A_{test} / A_{Control}) \times 100$$

(A_{test} = Absorbance of test sample)

($A_{Control}$ = Absorbance of Control)

The IC_{50} value of the compound MO5E3-a (2H-1-Benzopyran-2-one, 4-hydroxy) was found to be 58.84 ± 0.110 (µg/ml). Treatment with the purified compounds induced morphological changes characteristic of apoptosis, including cell retraction, rounding, and granulation, as shown in Figure 12.

The IC_{50} value from the MTT assay was higher than the MIC values for antibacterial activity, indicating that the compound is selectively antibacterial at concentrations below its cytotoxic threshold. The favourable Selectivity Index highlights the compound's therapeutic potential and safety margin.

Antibacterial activity was assessed exclusively using the autobiography diffusion assay. The coumarin derivative produced clear and measurable zones of inhibition against the tested pathogenic bacteria, demonstrating potent antibacterial action. Because the zones of inhibition were observed at concentrations much lower than those causing cytotoxicity in the MTT assay, the compound shows selective antibacterial behaviour relative to mammalian cell toxicity. This suggests a favourable safety margin and therapeutic potential even without MIC determination.

The present study primarily focused on isolation, characterization, and preliminary bioactivity evaluation. Therefore, mechanistic assays were not included. However, based on established coumarin pharmacophore behaviour, the compound's antibacterial activity may involve inhibition of bacterial DNA gyrase/topoisomerase, disruption of membrane integrity, or ROS-mediated mechanisms. Similarly, the observed cytotoxicity could be related to mitochondrial dysfunction, oxidative stress, or cell cycle interference. These mechanistic hypotheses are consistent with the behaviour of structurally similar coumarins but require further validation in dedicated molecular studies, which are planned for future work.

CONCLUSION

In the present study, 2H-1-Benzopyran-2-one, 3-amino-4-hydroxy, a coumarin derivative synthesized by marine bacteria *Bacillus subtilis*, was structurally characterized and evaluated for bioactivity. Coumarin represent a versatile class of natural compounds with well-known antimicrobial and anticancer activities. FTIR analysis confirmed key functional groups including hydroxyl, amino, and lactone carbonyl, indicating a typical substituted coumarin scaffold. GC-MS profiling supported the compound's identity through matching molecular ion peaks and fragmentation patterns. The observed activity of coumarin derivative in vitro indicates its potential as a lead compound for breast cancer treatment.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

TST, DRM, KG, KR and PSD designed the study. TST and DRM performed experiments and data analysis. PSD supervised the study. TST, KG and KR wrote the manuscript. PSD performed critical revisions. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available in the GenBank repository under the Accession number ON032864.

ETHICS STATEMENT

Not applicable.

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