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RESEARCH ARTICLE

Characterization of soil-derived *Bacillus subtilis* metabolites against breast cancer: In vitro and in silico studies

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Abstract

Breast cancer remains one of the most challenging and widespread cancers among women globally that warrants further investigations for novel agents to minimize side effects and disease recurrence. In this study, a soil bacterium was isolated and 16S rRNA nucleotide sequence homology and resultant phylogenetic tree analysis confirmed *Bacillus subtills* (CP020102.1). The crude extract of bacterial strain showed cytotoxicity (cell viability 63.1%) against human breast cancer cell line MCF-7 through MTT assay followed by further analysis through preparative HPLC showing 29 active fractions. Among all fractions, fraction 16 demonstrated cytotoxic potential as low as 54.03%. The spectral data of LC–MS, NMR and FTIR identified the bioactive compound as isatin based bacterial metabolite (Z)-N'-(1-hexyl-2-oxoindolin-3-ylidene)-4-methylbenzenesulfonohydrazide. This bioactive compound upon in silico docking analysis with HER2 showed a binding energy of -8.8 kcal/mol. ADMET calculations determined the pharmacokinetic behavior of the compound revealing acceptable distribution and absorption profiles. The drug-likeness of the compound was confirmed based on the Lipinski rule with zero violations. Molecular dynamic simulation (MDS) was also performed for 100 ns and a stable RMSF plot was observed. The results strongly propose that the identified compound has immense potential to be considered a drug candidate to develop further against human breast cancer.

Keywords *Bacillus subtilis* · Secondary metabolite · Anticancer activity · MCF-7 · Molecular docking

Abbreviations

MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
HPLC	High Performance Liquid Chromatography
LC-MS	Liquid Chromatography-Mass Spectrometry
FTIR	Fourier Transform Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance Spectroscopy
HER2	Human Epidermal Growth Factor Receptor 2

1 Introduction

Breast cancer remains the most prevalent type of cancer among women worldwide (Akbari et al. 2011; Houghton and Hankinson 2021), with its occurrence in 157 out of 185 countries (Arnold et al. 2022). The World Health Organization has reported 2.3 million new cases and 0.67 million mortalities globally in 2022, with almost half of all cases

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occurring in women associated with no specific risk factors except gender and age. Breast cancer is considered a pressing health concern as it is the leading cause of death from cancer in women (Trayes and Cokenakes 2021; Zhang et al. 2024).

Numerous receptors are associated with the development of breast cancer. In about 30% of breast cancer tumors, Human epidermal growth factor receptor 2 (HER2) is overexpressed and is responsible for the aggressiveness of tumors resulting in lower survival chances (Mitri et al. 2012; Schettini et al. 2020). The most common mode of treatment for breast cancer is chemotherapy, which has the potential to cause both acute and chronic side effects, from short-term discomfort to long-term issues that may emerge years after the treatment is received (Partridge and Winer 2004; Faruk 2021). These undesired side-effects and toxicity caused to normal cells by conventional treatment options like radiotherapy, chemotherapy, targeted immunotherapy or even surgery, can be avoided by the use of natural compounds from natural sources that offer broad-spectrum benefits with minimal to no side effects (Caicedo et al. 2012; Franzoi et al. 2021).

It is reported that about 13,000 natural compounds, each possessing different therapeutic and pharmacological characteristics, have been isolated from bacterial sources (Mohan et al. 2022). The vast diversity of bioactive secondary metabolites produced by bacteria has been known and valued for over a century (Tyc et al. 2017). Many of these metabolites have been reported as anti-cancer agents against different types of human cancers (Kennedy et al. 2015; Ali et al. 2016; Osama et al. 2022). Some of the prominent bacterial derived anti-cancer compounds currently in clinical use are Bleomycin (Chen et al. 2020; Shaikh et al. 2023), Dactinomycin (Kyrgiou et al. 2006; Liu et al. 2019), Doxorubicin (Thorn et al. 2011; Zhang et al. 2021), Carfilzomib (Gay et al. 2021; Burke et al. 2022). Among such compounds Ixabepilone, a bacterial metabolite (Mohan et al. 2022), is being used in the treatment of breast cancer (Cristofanilli 2012; Li et al. 2017; Azizah et al. 2024).

Numerous effective anticancer agents, e.g. sunitinib and semaxanib, consist of an isatin scaffold, signifying that isatin is an active moiety for developing novel anticancer agents (Alanazi et al. 2023). Many secondary metabolites that contain isatin moiety are obtained from bacterial sources (Wu et al. 2015; Ayuningrum et al. 2019; Triningsih et al. 2023).

The genus *Bacillus* produces secondary metabolites with antiviral (Abdel-Nasser et al. 2024; Salazar et al. 2023), antibiotic (Tran et al. 2022; Caulier et al. 2019), anti-cancer (Aimaier et al. 2023; Shao et al. 2021) potential. *Bacillus subtills* is commonly found in soil and has been known to produce bioactive compounds of various chemical nature, having linear or cyclic branching structures, which are particularly significant for their anti-tumor potential (Kaspar et al. 2019; Dan et al. 2021). Different bioactive compounds have been sourced from *Bacillus subtills* that showed cytotoxicity towards breast cancer cells and were subjected to molecular docking analysis with HER2 protein of breast cancer, demonstrating significant binding energies (Kumari and Ram 2021).

This study aimed to identify the anticancer potential and structure of bioactive secondary metabolites extracted and purified from the culture medium of *Bacillus subtills* by in vitro and in silico computational analysis.

2 Materials and methods

2.1 Sample collection and isolation of bacteria

A total of 30 soil samples were collected from various locations in Lahore, Pakistan, focusing on the upper soil layer where bacterial colonies are most abundant (Mishra 2013). Roughly 10 g of soil from each site was collected with the help of a sterile spatula and containers. Soil suspensions were prepared by mixing 1 g of each soil sample 10 mL of distilled water. Furthermore, by employing tenfold serial dilution, 1 mL of the suspension was diluted in 9 ml of distilled water stepwise up to 10^{-6}. (Kannan et al. 2018). The medium used in this research was nutrient agar (5.0 g/L peptone, 5.0 g/L NaCl, 1.5 g/L beef extract, 1.5 g/L yeast extract, 15 g/L agar) (Abdulkadir and Waliyu 2012). Initially, the spread plate method was employed in which 100 µL from each dilution was spread onto nutrient agar plates with the help of a sterile glass spreader. Plates were incubated at 37 °C until bacterial colonies appeared. Pure bacterial cultures were obtained by the streak plate method and incubated at 37 °C (Uddin and Chowdhury 2022).

2.2 Inoculum development and production of secondary metabolites

The inoculum was prepared according to the protocol described by (Thomas et al. 2011). Single and distinct colony from each bacterial pure culture was inoculated into 5 mL of Nutrient Broth (5.0 g/L peptone, 5.0 g/L NaCl, 1.5 g/L beef extract,1.5 g/L yeast extract) having pH 7.0 ± 0.2 and incubated in shaking incubator at 120 rpm and 37 °C for 24 h. Upon reaching adequate turbidity, indicating bacterial growth, the inocula were scaled up by transferring them into 20 mL of fresh medium. This production medium was incubated under identical conditions as previously described for 96 h. After it reached its maximal growth (OD_{600}: 2.5) (Numan et al. 2022), differential centrifugation was employed in which the culture

broth was centrifuged for 15 min at 3000 rpm (1,006 xg) using a low-speed bench top centrifuge with the rotor radius of 10 cm at room temperature (~ 25 °C) to obtain clear supernatant.

2.3 Extraction of secondary metabolites

The extraction of metabolites was carried out by the liquid–liquid extraction method, as described by (Burianek and Yousef 2000). In this method, equal amounts of supernatant and a freshly prepared mixture of chloroform–methanol (2:1 v/v) were vortexed in a sterile Eppendorf and centrifuged at 16,100 g for 5 min at 4 °C to separate the phases. The separated polar phase (upper layer) was transferred to a new sterile falcon without disturbing the rest of the content. The extracts were concentrated using rotary vacuum evaporator under reduced pressure. In the next step, the concentrated extracts were dried at 60 °C in a hot air oven. The final dried crude extract was stored at – 20 °C until further use.

2.4 Primary screening for cytotoxic activity

The cytotoxic potential of the bacterial extracts was evaluated using the MTT (3-(4,5-Dimethylthiazol-2-yl)−2,5-Diphenyltetrazolium Bromide) assay (Mosmann 1983) against the MCF-7 human breast cancer cell line. Cells were seeded at a density of 1×10^4 cells per well in a flat bottom 96-wellplate, with the cell culture media consisting of 88% Dulbecco's Modified Eagle's Medium (DMEM), 10% Fetal Bovine Serum (FBS), and 2% Penicillin–Streptomycin (Penstrep) per 100 mL of total medium. The cells were incubated at 37 °C in a humidified 5% CO_2 incubator for 24 h. After the incubation period, the culture medium was replaced, and cancer cells were treated with 20 µL bacterial extracts (100 µg/ml dissolved in DMSO) for an additional 24 h at 37 °C. Following treatment, 10 µL of MTT solution (5 mg/mL) was added to each well, and the plate was incubated for another 4 h at 37 °C. After incubation, the medium was carefully removed, and the resulting formazan crystals were solubilized in 50 µL of DMSO per well. Absorbance was measured at 570 nm using an ELISA plate reader to determine cytotoxicity (Akbarizare et al. 2020). The results were expressed as a percentage of viable cells compared to controls. Cell viability was calculated using the following formula (Sebola et al. 2020).

$$\%\ Cell\ viability = \frac{(E_a - B_a)}{(C_a - B_a)} \times 100,$$

where E_a is the absorbance of the extract, B_a is the absorbance of the blank, and C_a is the absorbance of the control.

2.5 HPLC fractionation

Crude extracts exhibiting the highest anticancer activities were subjected to preparative High-Performance Liquid Chromatography (HPLC) for fractionation and were collected based on their retention times (RT) (Rajivgandhi et al. 2018). The analysis was performed with Agilent Technologies 1260 Infinity II HPLC system equipped with a Diode Array Detector (DAD). A C-8 column (5 µm, 250 mm × 4 mm) was used with methanol as the mobile phase. The injection volume was set to 20.0 µL, with a flow rate of 1.0 mL/min. The column temperature was maintained at 40 °C, and detection was carried out at a wavelength of 210 nm. All the fractions were collected in sterile falcons, lyophilized, and stored at – 20 °C until further use. The retrieved purified fractions were further subjected to secondary screening (30 µg/mL) for their cytotoxic effect against MCF-7 cell lines.

2.6 Spectral studies and structure elucidation

^{1}HNMR (nuclear magnetic resonance) spectra of bioactive secondary metabolite was recorded at 300 MHz and ^{13}C NMR at 75 MHz on (Bruker Model: Avance-II) spectrophotometer. All chemical shifts are expressed in ppm. $CDCl_3$was used as the solvent for NMR spectroscopy (Raghava Rao et al. 2017).

Fourier transform infrared (FTIR) spectrum of bioactive secondary metabolite was obtained using an FTIR spectrometer (model NICOLET 6700; Thermo-scientific). The spectra covered the infrared region 4000–500 cm^{-1}.

LC–MS analysis was performed using Agilent 6300 ion trap LC/MS systems. Chromatographic separation was achieved with an isocratic mode with acetonitrile (ACN) as the mobile phase at a flow rate of 0.5 mL/min. MS detection was carried out using electrospray ionization (ESI). The mass scanning range was up to 1000 m/z and mass detection was performed in the negative ion mode. Chemstation software was used for data processing. Thin-layer chromatography (TLC) was performed using Merck silica gel 60F254 pre-coated aluminum plates with n-hexane ethyl acetate (4:6) as the mobile phase. UV-active compounds were visualized under UV light at 254 and 365 nm, while different staining agents were applied for UV-inactive compounds.

2.7 Molecular characterization

The bacterial isolate was identified through 16S rDNA sequencing by using universal primer pairs (Ashfaq et al.

2021). Sequencing primers 785F (5'-GGA TTA GAT ACC CTG GTA-3') and 907R (5'-CCG TCA ATT CMT TTR AGT TT-3') were used for the sequencing process. PCR amplification was performed using primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3'). The phylogenetic relationship of the isolate was determined based on sequence homology using the Neighbor-Joining method (White et al. 1990; Bellemain et al. 2010).

2.8 *In silico* studies

Various in silico studies, including 3D structure development, prediction of pharmacokinetics and drug likeness, molecular docking and molecular dynamic simulation studies were performed for the validation of selected bioactive metabolite and its interactional behavior towards breast cancer receptors.

2.8.1 Structure determination of bioactive metabolite

The general molecular and formula based parent structure of the selected compound determined through NMR was further used to generate its canonical SMILES by using the online tool Cheminfo (https://www.cheminfo.org/flavor/malaria/Utilities/SMILESgeneratorchecker/index.html).

The generated SMILES was further used to draw the 2D structure in SDF file format through Chemdraw, an online chemistry server (https://revvitysignals.com/products/research/chemdraw) (Cousins 2011).

0.3D representation in the ball and stick model was determined by the Pymol (http://www.pymol.org/pymol) software and saved in PDB file format (Schrödinger and DeLano 2020). This PDB file format of the compound was used as ligand compound for further studies.

2.8.2 Prediction of pharmacokinetics and druglike properties

Druglike properties of the selected compound were predicted by an online tool SWISSADME (http://www.swissadme.ch/) (Daina et al. 2017).

2.8.3 Selection and retrieval of oncogenic receptor proteins

Different breast cancer receptor proteins such as Estrogen Receptor Alpha (ERα), Human Epidermal Growth Factor Receptor 2 (HER2), Progesterone Receptor (PR), VEGFR2 (Vascular Endothelial Growth Factor) and Polo-like Kinase (PLK1) were selected based on their potential role in disease and occurrence of progression (Gam 2012).

3 dimensional crystallized structures of the all-mentioned proteins were downloaded from RCSB PDB database (https://www.rcsb.org/), having unique PDB ID (ERα−1ERE, HER2-3PP0, PR-1A28, VEGFR2-2VPF, PLK1-3D5U) (Burley et al. 2021). All these receptor proteins were used as target proteins against above mentioned bioactive metabolite.

2.8.4 Preparation of target proteins and ligand compound

Discovery Studio tool (https://discover.3ds.com/discovery-studio-visualizer-download) was used to prepare target proteins by removing all the attached water molecules, ligands, metals and ions by San-Diego (2021). PyMol viewer software was employed to view and save prepared targets in PDB file formats. Ligand (bioactive metabolite) was also saved in the same PDB file format and was used for molecular docking analysis with target proteins.

2.8.5 Molecular docking analysis

All the PDB files of target proteins were converted to PDBQT files to run docking by Autodock Vina software of MGL tools version 1.5.7 (Trott and Olson 2010). Hydrogen molecules and kollmann charges were added for the energy refinement and these refined PDB structures were converted to PDBQT files by Vina software. The ligand (bioactive compound) was also prepared by adding torsion angles and the torsion root was adjusted. Various commands were made from the command prompt to run the docking experiments for each target. The grid dimension was adjusted at 1.000 Å spacing and numbers of points of all three dimensions were set as 100. The grid values for X, Y, and Z coordinates were 21.407, 31.289 and 14.676, respectively. PyMOL viewer software and Discovery Studio tool https://discover.3ds.com/discovery-studio-visualizer-download) were used to analyze the docking results. Top receptors protein was selected and used for simulation studies, selection was made on binding energy and residual interactions.

2.8.6 Molecular dynamic simulation analysis

The best docked complex (HER2) was evaluated further by molecular dynamic simulation studies to check the interactional behavior of the docked complex in physiological conditions (Huang et al. 2023). Desmond Software, LLC v3.6 module, version 2019, was used to perform MD simulation analysis, which was carried out for 100 ns. Wizard in Maestro tool was employed for the preparation of target proteins constant temperature of 300 k and 1 atm pressure with OPLS-2005 all atoms force field.0.12 M NaCl ions were added to maintain the ionic concentration at neutral charges. Following a successful

100 ns simulation, the results were analyzed, and the two most common metrics, root mean square deviation (RMSD), root mean square fluctuations (RMSF), were used to evaluate the stability of protein–ligand docked complexes (Ayan et al. 2017).

2.9 Statistical analysis

All the experiments were performed in triplicate. One-way ANOVA was used to test the significant ($p \leq 0.05$) differences between means of experimental values by using SPSS program version 16.

3 Results and discussion

3.1 Bacterial isolates

Out of 30 soil samples, 200 bacterial isolates were obtained. Soil samples proved very effective sources of bacteria that have the potential to produce cytotoxic metabolites of a diverse chemical nature (Tyc et al. 2017; Gislin et al. 2018). Bacterial genera like *Bacillus* (Aimaier et al. 2023; Shao et al. 2021; Shalini et al. 2024), *Streptomyces* (Hamed et al. 2024; Sethi et al. 2024; Nascimento Chaves et al. 2024), *Salmonella* (Aganja et al. 2022; Kalia et al. 2022), *Escherichia* (McCoy et al. 2021; Nakkarach et al. 2021), *Pseudomonas* (Sereena and Sebastian 2020; Subbiah et al. 2024), and *Clostridium* (Yaghoubi et al. 2022; Liu et al. 2023) have been reported to produce anticancer metabolites.

3.2 Extraction of secondary metabolites

The usage of a chloroform-containing solvent system for the extraction of bacterial secondary metabolites is supported by various previous studies (Burianek and Yousef 2000; Anantha et al. 2016; Abdelaziz et al. 2022) in which it was verified that the chloroform based organic solvent system may improve the extraction of bacterial compounds by many folds.

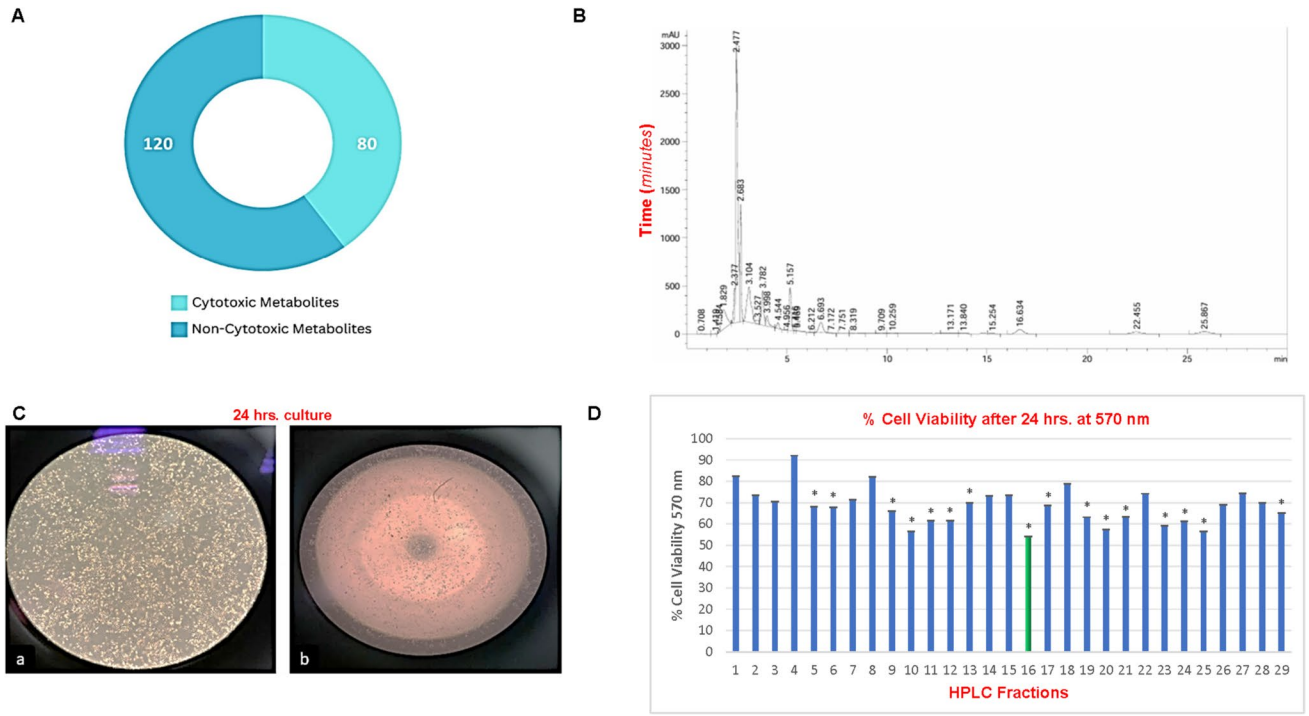


Fig. 1 **A** Distribution of cytotoxic and non-cytotoxic metabolites following primary screening against MCF-7 cells. **B** HPLC chromatogram of the crude cytotoxic extract 74 following primary screening. The chromatogram shows 29 distinct peaks between 0 to 26 min at 210 nm. **C** Percentage cell viability (%) at 570 nm for each HPLC fraction. Fraction 16 (green bar) exhibited a mean absorbance of 0.41, corresponding to 54.03% viability. Data were analyzed by one-way ANOVA ($p \leq 0.05$). Asterisks (*) indicate statistically significant differences from the control. **D** Breast cancer cells before and after 24-h treatment with fraction 16

3.3 Primary screening for cytotoxic activity

Out of 200 bacterial extracts, 80 were cytotoxic against MCF-7 cells (Fig. 1A). Three extracts, 76, 27, and 74, showed cell viability values of 64.8%, 63.7% and 63.1%, respectively. It has been confirmed in recent studies that bacterial metabolites exhibit cytotoxic properties against different types of cancers (Laliani et al. 2020; Behzadi et al. 2021; Elmanama et al. 2020).

3.4 HPLC fractionation

The crude extract demonstrating the highest cytotoxic activity (extract 74) in primary screening, was subjected to fractionation using preparative HPLC resulting in the identification of ***29 distinct fractions***, each corresponding to a unique peak in the chromatogram obtained at 210 nm with retention times ranging from 0.708 to 25.867 min (Fig. 1B). Notably, Peak 6 (tR: 2.477 min) exhibited the highest percentage area (38.74%), suggesting it is the most abundant component in the extract. Other significant peaks include Peak 7 (tR: 2.683 min) and Peak 8 (tR: 3.104 min), with percentage areas of 11.43% and 12.00%, respectively. These fractions are expected to contain the bioactive compounds that were responsible for cytotoxicity induction during primary screening. These fractions were further subjected to secondary screening.

Previous research has shown that HPLC fractionation is a well-established method for isolating bioactive compounds from complex extracts of both bacteria and plants. For instance, the crude extract of *Bacillus sp*. strain B29 produced 17 fractions (Sihem et al. 2011), and the extracts of Glycyrrhiza glabra produced 20 fractions (Rahman et al. 2018). Chalasani et al. (2015) and Shalini et al. (2024) performed RP-HPLC on *Bacillus subtilis* and *Bacillus cereus* extracts, respectively, and obtained various peaks between the retention time of 5 to 30 min.

3.5 Secondary screening for cytotoxic activity

Among all the fractions tested, fraction 16 (30 µg/ml in DMSO) showed the lowest cell viability of 54.03%, indicating it had the most significant cytotoxic effect (Fig. 1C). This suggests that Fraction 16 contains potent bioactive compounds that significantly reduce cell viability (Fig. 1D), making it the most promising candidate for further investigation. The cytotoxic activity of a bioactive compound increases when used in a purified form. For instance, in another study, a bioactive metabolite extracted from culture supernatant of *B. vallismortis* BIT-33 was purified by Jeong et al. (2008), and the compound showed direct cytotoxic and apoptotic effects on colon cancer cells.

Other fractions, such as Fraction 25 (56.38% cell viability) and Fraction 9 (65.89% cell viability), exhibited notable cytotoxic effects but were less effective than Fraction 16. The observed decrease in cell viability confirms that these fractions have substantial cytotoxic potential and warrant additional study to elucidate their active components and predictions of their drug-likeness abilities.

3.6 Identification of isolate 74 using 16S rRNA profile

Isolate 74 was selected and identified as *Bacillus subtills* with 99% similarity and then deposited at GenBank with accession number CP020102.1. Figure 2A shows the phylogenetic tree of *Bacillus subtills* CP020102.1

3.7 LCMS data of compound

The LC–MS analysis of the bioactive compound from Fraction 16 and the protonated molecular ion peak $[M+H]^+$ was observed at m/z 400.12 (Fig. 2B), indicating that the compound's molecular weight is 399.12 amu. Fragmentation peaks were also observed at m/z 365.8 and m/z 507.5, likely representing the loss of smaller functional groups or possible adducts with solvent molecules. The fragment at m/z 365.8 corresponds to a neutral loss of approximately 34 amu, potentially from side-chain cleavage. The peak at m/z 507.5 may indicate the presence of adducts, possibly due to interaction with matrix ions or solvent components.

The molecular weight of 399 Da excludes the possibility of metabolites being iturins, fengycins, and surfactins known as *Bacillus* lipopeptides and have molecular weight between 1,000–1,200 Da (Ongena and Jacques 2008). Also, it is unlikely to be a part of the class II or III bacteriocins because the latter include larger peptides and protein complexes, many of which have molecular weights over several thousand Da (Wang et al. 2024). Due to its lower molecular weight and anticancer activity, this compound may contain an aromatic ring, similar to other biologically active bacterial metabolites, including phenazines and polyketides, which contain aromatic rings and are target specific and bioavailable (Pereira et al. 2009; Laursen and Nielsen 2004).

3.8 FTIR spectroscopy analysis

The FTIR spectrum (Fig. 2C) of the bioactive compound from Fraction 16 provides detailed insights into its molecular structure. Signals at specific frequencies suggest the presence of various functional groups: 3175 cm^{-1} indicates N–H stretching vibrations of secondary amides, and 2959 cm^{-1} corresponds to Csp^2–H stretching, which is characteristic of aromatic rings. Peaks at 2935 and 2869 cm^{-1} indicate asymmetric and symmetric stretching vibrations,

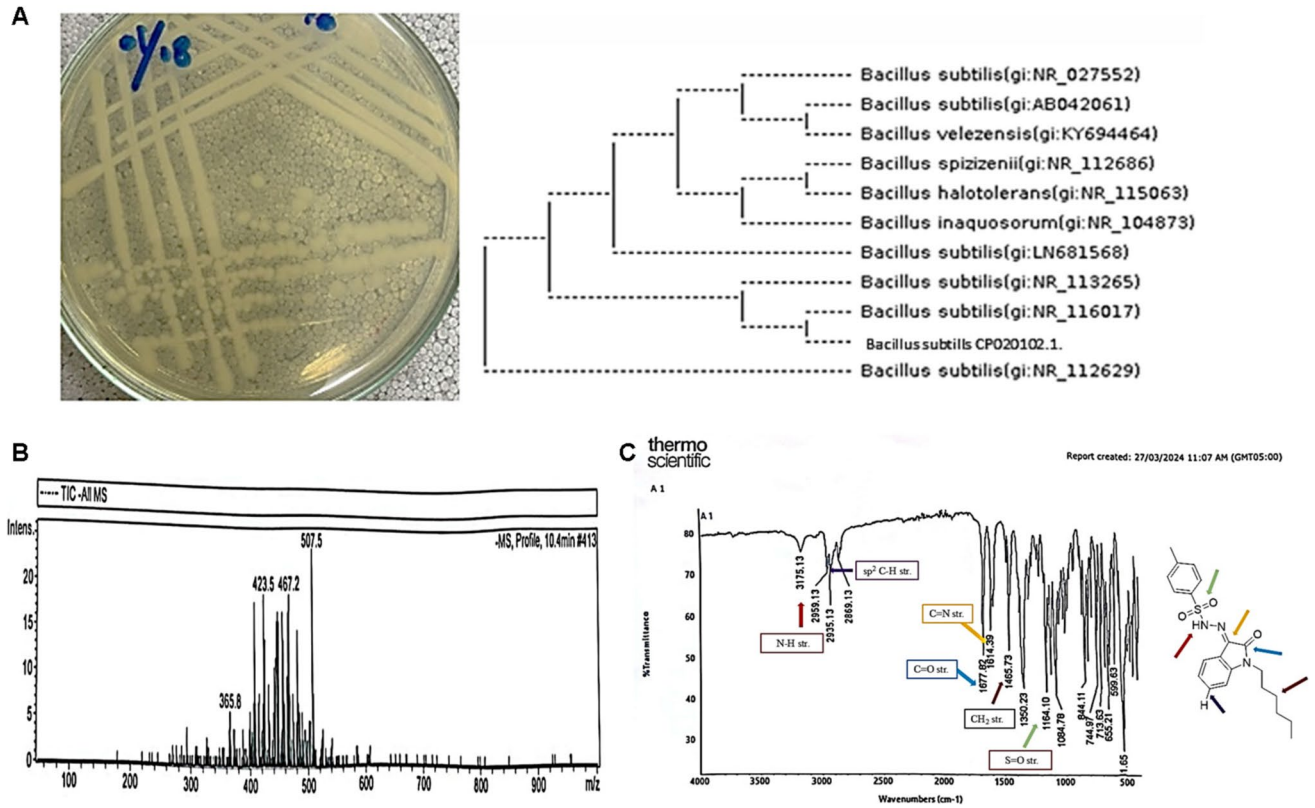


Fig. 2 **A** Phylogenetic tree of *Bacillus subtills* CP020102.1. **B** LC–MS Spectrum of Bioactive Compound from Fraction 16. **C** FTIR spectrum of the bioactive compound from Fraction 16, highlighting key functional groups including amides, aromatic rings, methyl groups, and sulfone linkages

respectively, of a sp^3 hybridized methyl group. Weak absorptions between 2000 and 1677 cm^{-1} suggest overtone and combination bands that help determine ring substitution patterns. A strong absorption at 1667 cm^{-1} is attributed to the carbonyl stretching of an amide or cyclic amide. Additional signals at 1614, 1601, and 1465 cm^{-1} correspond to C = N and C = C stretching and Csp^3–H bending, with the peak at 1465 cm^{-1} indicating the presence of a methylene group. Absorptions at 1350 and 1164 cm^{-1} indicate S = O group stretches, and bands at 1233 and 1033 cm^{-1} suggest a phenyl alkyl ether linkage. Signals at 714 cm^{-1}, 844 cm^{-1}, 713 cm^{-1}, and 655 cm^{-1} provide further insights into CH_2 bending and aromatic substitution patterns. The FTIR spectrum confirms the presence of amide, aromatic ring, ether linkage, and aliphatic methylene functionalities in the bioactive compound of Fraction 16.

Several anticancer *Bacillus* derived metabolites are reported that are heterocyclic and contain sulfur and nitrogen (Zhang et al. 2022), for instance, Bacillamide A is produced by Bacillus sp. SY-1 (Jeong et al. 2003), Bacillamide B and C are produced by *Bacillus endophyticus* (Socha et al. 2007) and Bacillamide F is produced by *Bacillus atrophaeus* (Zhang et al. 2022). Furthermore, the presence of nitrogen and sulfur in a heterocyclic compound studied for multi-target pharmacology, especially in cytotoxic applications, may increase therapeutic efficiency in cancer (Carradori et al. 2022; Drakontaeidi et al. 2024).

3.9 ^{1}H NMR analysis

In the ^{1}H NMR spectrum of the bioactive compound from Fraction 16 (Fig. 3A), the appearance of a signal in the most de-shielded region at 12.60 ppm with the integration of one proton gave the evidence that one hydrogen attached with most de-shielded species such as aldehyde or amide (-NH-C = O). The triplet with the integration of two protons at 3.69 ppm due to the two neighboring protons and high chemical shift indicated -CH_2 group is further attached with heteroatom as (O-CH_2 or N-CH_2). The singlet having integration of three protons at 2.43 ppm indicates the proton of methyl with no neighboring proton attached with sp^2 hybridized carbon. The multiplet ranging from 1.70–1.63 ppm with the integral of two protons indicates the presence of neighboring protons of different environments in the sample as (NH-CH_2-CH_2-CH_2). The multiplet in the aliphatic region with the integral of six protons at 1.37–1.28 ppm

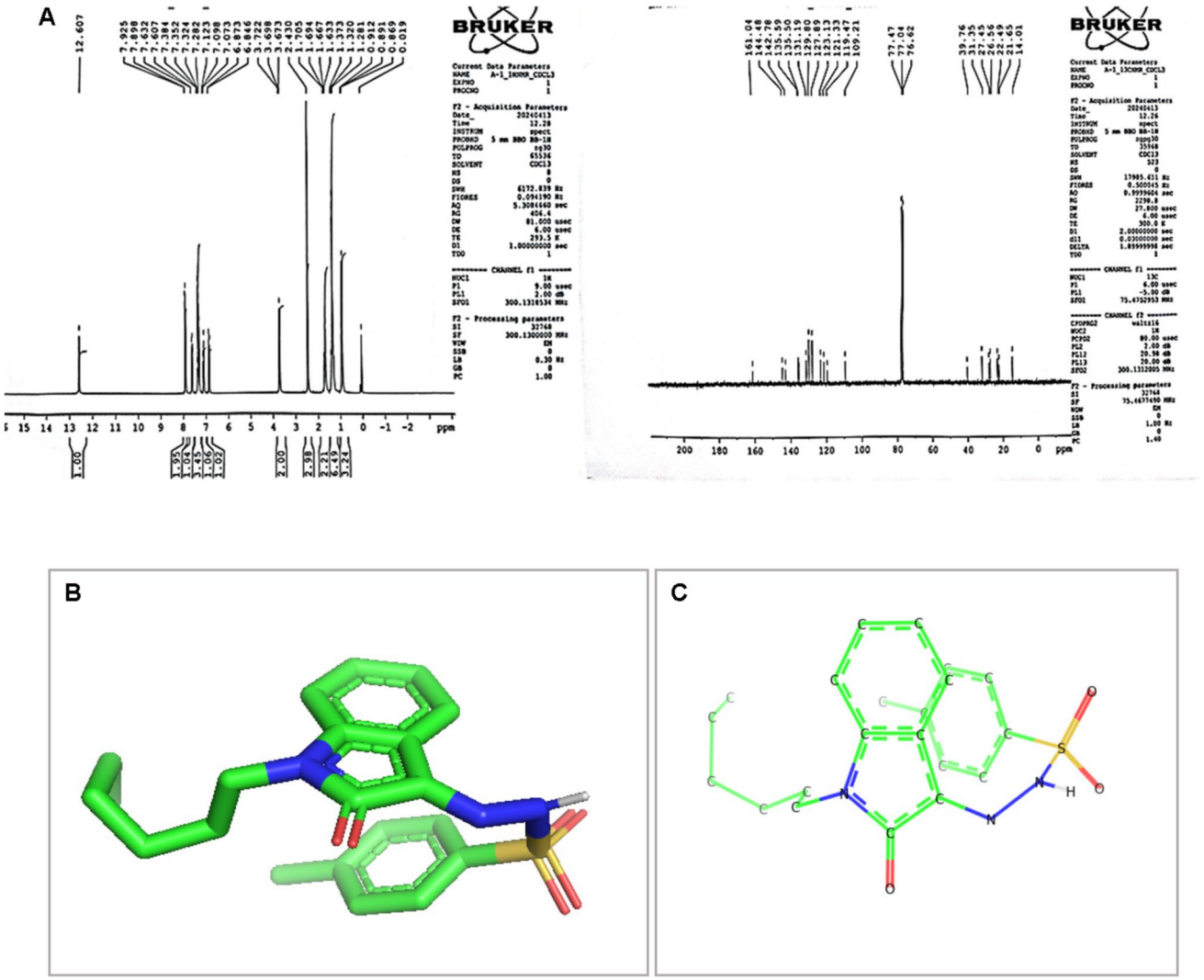


Fig. 3 **A** ^{1}HNMR and ^{13}C NMR spectrum of the bioactive compound from Fraction 16. **B** 3D structure representation of bioactive compound. **C** 2D Structure representation of compound with molecular symbol

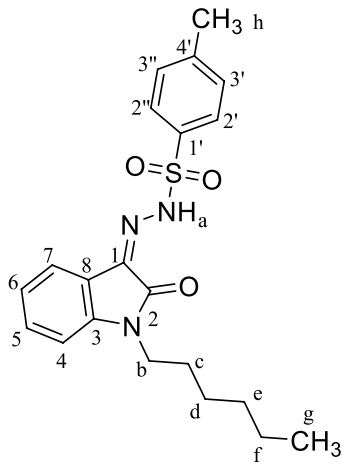
gave evidence of the presence of a long aliphatic chain as (CH_2-CH_2-CH_2-CH_2-CH_2). The signals of eight aromatic protons appeared in the range of 7.92 to 6.84 ppm indicating the presence of two substituted benzene rings.

3.10 ^{13}C NMR analysis

The ^{13}C NMR of the bioactive compound from Fraction 16 demonstrated the most de-shielded signal at 161.0 indicating the presence of any carbonyl. The appearance of a signal at 135.5 ppm for carbon indicates the imine linkage (C = N), it may also indicate the formation of imine linkage. The signal appeared at 39.7 ppm indicating the carbon directly attached to heteroatom i.e., N, O, S etc. The signals at 31.3, 27.4, 26.5, 22.4, 21.6 ppm and 14.0 ppm showed the presence of long aliphatic chain of carbons, respectively. The signals of aromatic carbons are in the range of 144.4 to 109.2 ppm. Overall, from the ^{13}C NMR results it was confirmed that seven carbons present in the aliphatic region, one signal of carbon carbonyl, and signals of eleven carbon in the aromatic region in structure of unknown compound.

NMR results shows that the structure contains isatin moiety. Isatin is reported to be isolated from natural sources including plants (Speranza et al. 2020; Marcelo et al. 2019) and microorganisms (Gil-Turnes et al. 1989) specifically bacteria (Shaaban et al. 2016; Grafe and Radics 1986).

Spectral and physical characterization of bioactive compound



Appearance: Yellow solid; Melting Point: 220–225 °C; Rf: 0.47 (n-hexane: ethyl acetate, 6:4); FT-IR ῡ (cm−1); 3175 (N–H stretch of amide), 2959 (Csp2–H), 2935, 2869 (Csp3–H), 1677 (lactam C = O), 1614 (imine C = N), 1465 (–CH2– bending); 1H NMR (300 MHz, CDCl3-d): δ (ppm): 12.60 (1H, s, NH-a), 7.91 (2H, d, H-2′, 2′′), 7.61 (1H, d, H-7′), 7.38–7.28 (3H, m, H-3′, 3′′, 4), 7.09 (1H, t, H-6), 6.85 (1H, d, H-5), 3.72–0.86 (13H, H-b,c,d,e,f,g) aliphatic protons; 13C NMR (75 MHz, CDCl3):δ (ppm): 161.0 (C-2), 131.1 (C-3′), 144.4, 142.7, 135.5, 129.8, 127.8, 123.1, 121.3, 119.4, 109.2 (aromatic carbons); EI-MS (m/z): 507.5[M-107]−, 467.2, 423.5, 365.8;

3.11 In silico *analysis*

3.11.1 3D Structure determination of compound for

The structure of bioactive compound was determined and PDB file was generated by computational tools. Figure 3B and C shows the 2D and 3D PDB structure of bioactive compound prepared for docking analysis.

3.11.2 Prediction of pharmacokinetics

The bioactive compound was analyzed for pharmacokinetics properties by Swiss ADME tool and mainly checked for Lipinski rule of 5 along with drug likeness properties. Results shows that bioactive compound fulfilled Lipinski rule of 5 and possess pharmacokinetics properties as well. According to this rule, a drug-like compound should have a molecular weight (MW) less than 500 g/mol, a log P less than 5, hydrogen bond donors less than 5, hydrogen bond acceptor less than 10 and rotatable bonds less than 10 (En-Nahli et al. 2023). Pharmacokinetics includes GI absorption and Blood Brain Barrier (BBB). Conclusively, it was found that the compound has drug-like properties and most of the predicted values lie within acceptable range (Table 1). Likewise, a study was reported where Swiss ADME tool was used to predict these properties and compound passed Lipinski rule of 5 along with other pharmacokinetics parameters were predicted to have drug like activity (Agour et al. 2022). Radar chart was also retrieved which shows that all drug properties all required physiochemical properties (lipophilicity, size, polarity, insolubility, instauration and flexibility) lies within the stability zone. Radar points with red line of the properties lie within optimal zone. The optimal zone shown by pink region (Fig. 4A). This is in reference with a study in which after fulfilling these six parameters within the pink region the compound was considered drug-like compound (Ritchie et al. 2011).

3.11.3 Docking analysis

Molecular docking results revealed that the compound showed good binding score with Human Epidermal Growth Factor Receptor 2 (HER2) i.e. −8.8 kcal/mol and various residues shows interactions towards HER2. The larger the negative value of binding energy, the stronger the affinity of the ligand with the receptor (Shamsee et al. 2019). A study has been documented that the docked complex with least binding energy considered as the most potential docking pose (Terefe and Ghosh 2022).

Moreover, docking results revealed that Thr862 amino acid residue made hydrogen bond with 3.25Å bond length represented by blue dashed line with target. Hence HER2 selected as top receptor target and used for further simulation studies. Docking interaction with high binding energy shows that compound may be predicted as anticancer compound against breast cancer (Fig. 4A, C and Table 2). Here the interaction with Thr amino acid was found significant also evident by a previous report regarding inhibitors

Table 1 Pharmacokinetics examination and Lipinski rule of 5 predictions

Physicochemical properties		Pharmacokinetics		Drug likeness	
M.F	$C_{21}H_{25}N_3O_3S$	GI absorption	High	LipinskiRule of 5	Fulfill
M.W	399.51 g/mol				
HA	28	BBB permeant	Low	Lipinski violations	0 violations
HBA	4				
HBD	1				

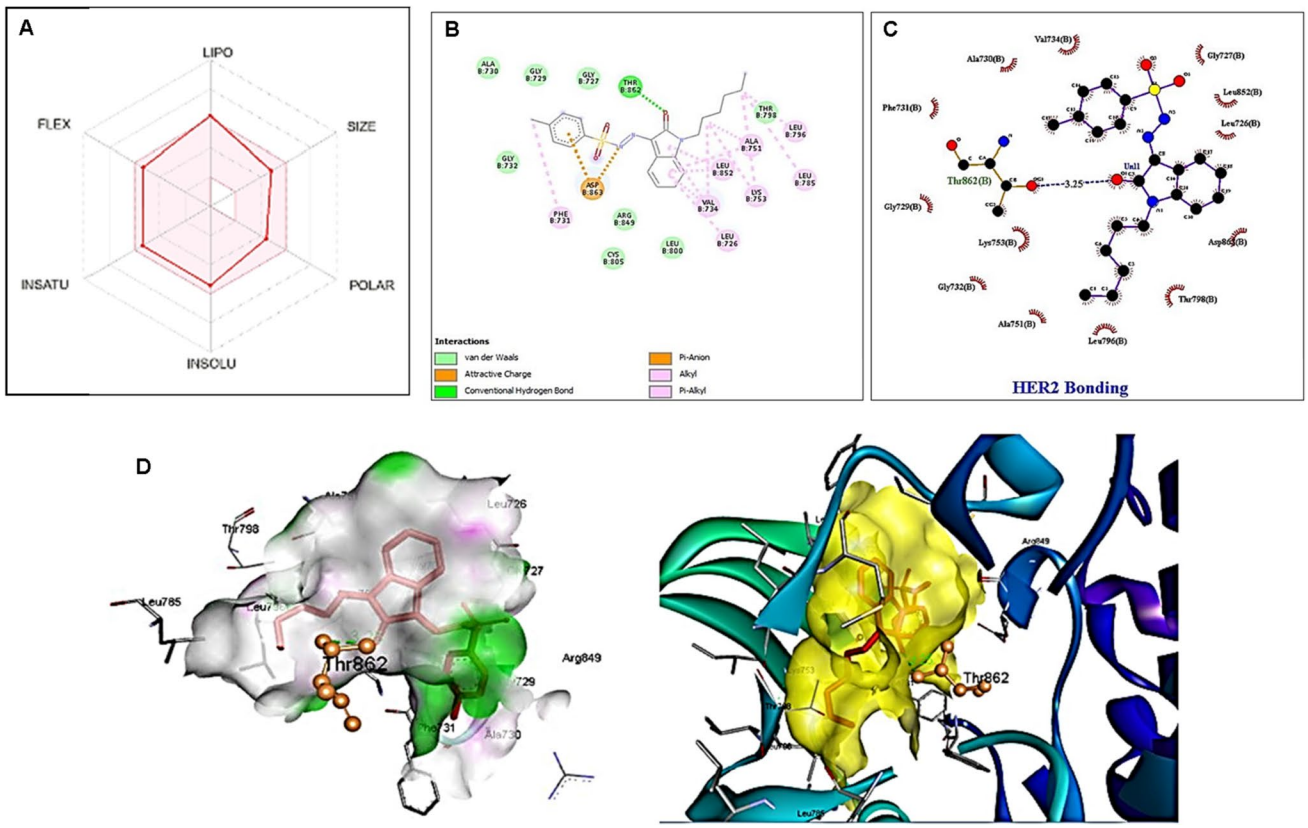


Fig. 4 **A** RADAR chart determination of the compound. **B** 2D interaction of docking analysis between ligand compound and Target protein HER2: Discovery studio image representation of docking results. **C** Ligplot image showing hydrogen bonding and hydrophobic interaction between compound and HER2. **D** 3D model of docked ligand with target protein

Table 2 All the binding Score of docking complexes between bioactive compounds and target cancer receptor proteins

Sr. no	TARGET PROTEIN	BINDING ENERGY (Kcal/mol)	INTERACTING RESUDUES	RMSD
1	Estrogen Receptor Alpha (ERα)	−8.4	Glu380, Val534, Pro535, Met528, Leu384, Lys531, Thr347,Met522, Leu536, Trp383, Ala350, Leu346, Leu525, Asp351, Leu354	0.000
2	**Human Epidermal Growth Factor Receptor 2 (HER2)**	**−8.8**	**Ala730, Gly729, Gly727, Thr862, Thr798, Leu796, Ala751, Leu852, Leu785, Lys753, Val734, Leu726, Leu800, Arg849, Cys805, Phe731, Gly732**	**0.000**
3	Progesterone Receptor (PR)	−8.6	His770, Trp765, Met692, Gly762, Phe818, Lys822, Val729, Trp732, Met759, Leu758, Arg766, Glu695, Gln725, Pro696, Ser728, Ile699, Gln815, Val698, Asp697	0.000
4	VEGFR2(Vascular Endothelial Growth Factor)	−8.5	Lys868, Asp814, Arg1027, Ile1025, His1026, Ile888, Ile892, Asp1046, Leu889, Glu885, Ile1044, Cys1045, Val898, Leu1019, Val899, Val916, Val848, Leu1035, Phe1047	0.000

development against HER2 and their findings shows that Asn850, Leu852, Thr862, Asp863, and Phe864 of HER2 catalytic site are the important interacting amino acid (AA) residues with their selected (Sait et al. 2020). 3D model of selected docked complex was also generated (Fig. 4D).

3.11.4 Molecular stability evaluation of docked complex between bioactive compound and HER2 receptor protein

MD simulation results showed the estimation of RMSD value on y-axis with respect to time in 100 ns on X-axis for Cα particles. Blue colored line represents the protein while

the pink colored lines represent RMSD evolution of the ligand. RMSD plots shows that complex gets stabilizes after 20 ns and little variations occurred after 35 ns and become unstable for 5 to 7 s only, equilibrates and stabilized till end. (Fig. 5A). Average RMSD values of the plot is 3.4 Å, which is quite acceptable. This is in agreement with a published study where a range of average RMSD values has been given and above-mentioned results falls in that range which shows that ligand fit to protein and remain stable at the binding site of the protein during the simulation (Riaz et al. 2024).

Root mean square fluctuations (RMSF) plot was also determined for simulation evaluation between target protein and ligand. This plot is used to analyze the residue wise fluctuations of the protein bounded with the ligand compound. Results shows that less fluctuations were observed during simulation and low RMSF values of both ligand and protein indicates that over all protein–ligand complex is stable (Fig. 5B and C). Most of the peaks are at lower level and green colored bars within the plot represent those residues which are in contact with ligand. Both the ligand and protein RMSF average values are 3.0 Å and 3.1 Å respectively and these values sows that complex used for simulation is highly stable as lower the value the more stability is present. A study has been published in which a docked complex of HER2 with a screened molecule represent and amino acids fluctuations found between RMSF: ~ 4.0–6.5 Å during the simulation process of 100 ns and their collectively results showed the stability of docking in the defined active site of HER2 (Balogun et al. 2022).

Most important simulation examination is protein ligand contacts which is also monitored during molecular dynamic simulation of HER2 with the bioactive compound to evaluate its stability. This graph shows how many and which type of bonding is present between ligand and target complex used for simulation studies. HER2 results revealed that almost 5 hydrogen bonds, various hydrophobic and ionic interactions present with ligand and target (Fig. 5D). Green bars represented that 5 hydrogen bonds are present which shows strong stable interaction. In accordance with in silico research where lead compound was identified computationally from *Enterococcus faecium* strains, it has been concluded that hydrogen and

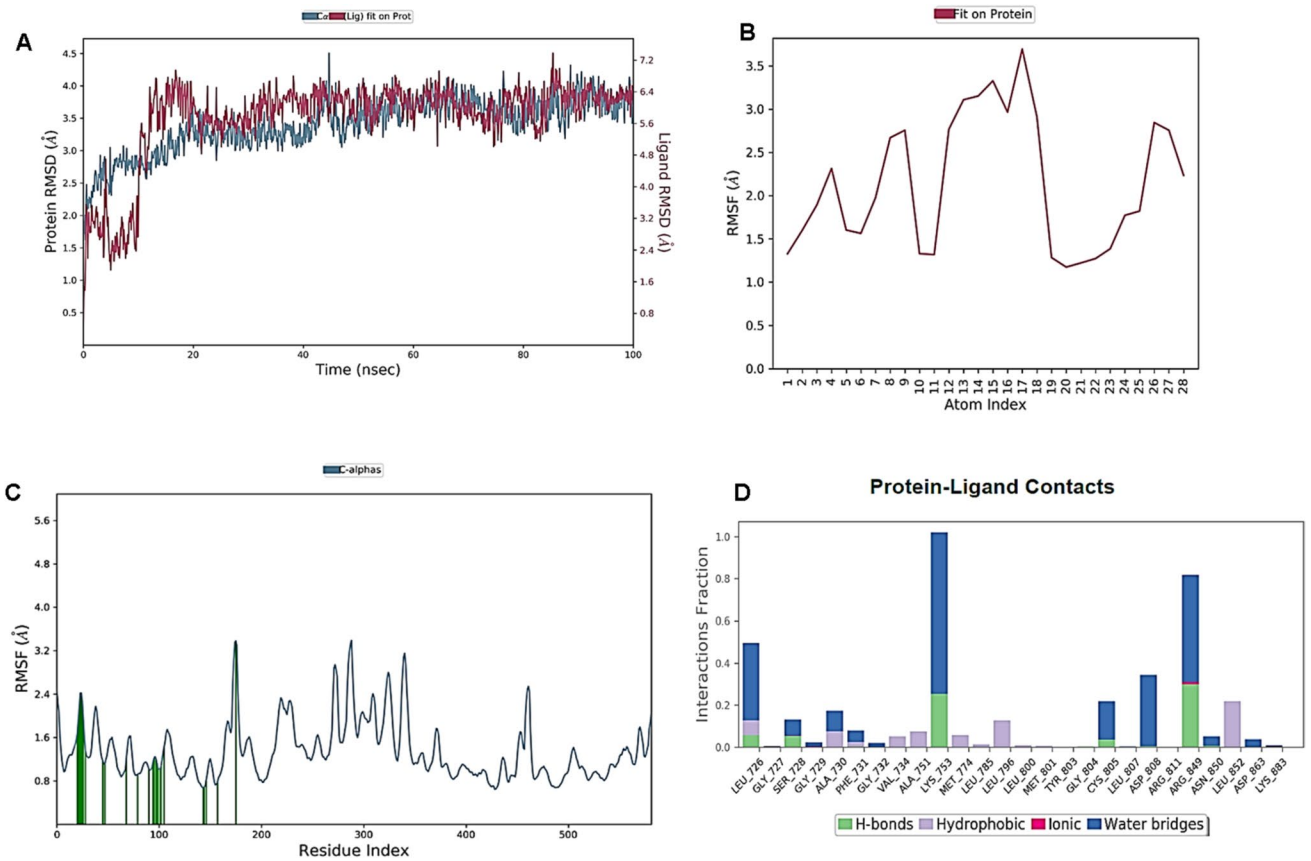


Fig. 5 **A** RMSD evolutions of docked complexes between the C-alpha atoms of HER2 protein and ligands in 100 ns simulation. **B** Residue wise Root Mean Square Fluctuations (RMSF) graph between HER2 protein and ligand in 100 ns simulation: RMSF plot obtained from Ligand RMSF values. **C** RMSF plot obtained from protein RMSF values. **D** Protein–Ligand Contacts between HER2 cancer receptor protein and ligand compound in 100 ns simulation

hydrophobic interactions are most important in MD simulations to determine ligand–proteins complex and their findings represents that Thr and Ser (Threonine and Serine) are the most significant residues in terms of H-bonds (Rasheed et al. 2021).

4 Conclusion

The conclusive findings shows that bioactive metabolite extracted from soil derived *Bacillus subtilis* was cytotoxic to human breast cancer MCF-7 and after in silico studies support these results as the bioactive metabolite showed promising binding energy of −8.8 kcal/mol against HER2. The dynamic simulation results of this HER2 docked complex shows that HER2 have stable interaction towards ligand, hence it was predicted that bioactive ligand compound can interact with human HER2 cancer protein and can be used as anti-cancer compound to treat breast cancer.

Authors contribution Ayesha Irfan Hashmi (AIH); investigations, data curation, data analysis, wrote first draft, Mehwish Iqtedar (MI); conceptualization, supervision, resources, project administration, manuscript editing and review, Hamid Saeed (HS); resources, data analysis, validation, manuscript editing and review, Nadeem Ahmed (NA); data curation, data analysis, validation, Roheena Abdullah (RA); data curation, investigation, manuscript editing and review, Afshan Kaleem (AK); investigations, software, data analysis, visualization, Muhammad Athar Abbasi (MAA); Investigations, data analysis, validation.

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Data availability Will be available on reasonable request to the corresponding authors.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

Ethics declaration Not applicable.

References

Abdelaziz AA, Kamer AMA, Al-Monofy KB, Al-Madboly LA (2022) A purified and lyophilized Pseudomonas aeruginosa derived pyocyanin induces promising apoptotic and necrotic activities against MCF-7 human breast adenocarcinoma. Microb Cell Fact 21(1):262. https://doi.org/10.1186/s12934-022-01988-x

Abdel-Nasser A, Badr AN, Fathy HM, Ghareeb MA, Barakat OS, Hathout AS (2024) Antifungal, antiaflatoxigenic, and cytotoxic properties of bioactive secondary metabolites derived from Bacillus species. Sci Rep 14(1):16590. https://doi.org/10.1038/s41598-024-66700-y

Abdulkadir M, Waliyu S (2012) Screening and isolation of soil bacteria for their ability to produce antibiotics. Eur J Appl Sci 4(5):211. https://doi.org/10.5829/idosi.ejas.2012.4.5.2011

Aganja RP, Sivasankar C, Senevirathne A, Lee JH (2022) Salmonella as a promising curative tool against cancer. Pharmaceutics 14(10):2100. https://doi.org/10.3390/pharmaceutics14102100

Agour MA, Hamed AA, Ghareeb MA, Abdel-Hamid EA, Ibrahim MK (2022) Bioactive secondary metabolites from marine Actinomyces sp. AW6 with an evaluation of ADME-related physicochemical properties. Arch Microbiol 204(8):537. https://doi.org/10.1007/s00203-022-03092-5

Aimaier R, Li H, Cao W, Cao X, Zhang H, You J, Zhao H (2023) The secondary metabolites of Bacillus subtilis strain Z15 induce apoptosis in hepatocellular carcinoma cells. Probiot Antimicrob Proteins 31:1–11. https://doi.org/10.1007/s12602-023-10181-4

Akbari A, Razzaghi Z, Homaee F, Khayamzadeh M, Movahedi M, Akbari ME (2011) Parity and breastfeeding are preventive measures against breast cancer in Iranian women. Breast Cancer 18:51–55. https://doi.org/10.1007/s12282-010-0203-z

Akbarizare M, Ofoghi H, Hadizadeh M, Moazami N (2020) In vitro assessment of the cytotoxic effects of secondary metabolites from Spirulina platensis on hepatocellular carcinoma. Egypt Liver J 10:1–8. https://doi.org/10.1186/s43066-020-0018-3

Alanazi AS, Mirgany TO, Alsfouk AA, Alsaif NA, Alanazi MM (2023) Antiproliferative activity, multikinase inhibition, apoptosis-inducing effects and molecular docking of novel isatin–purine hybrids. Medicina 59(3):610. https://doi.org/10.3390/medicina59030610

Ali HM, El-Shikh MS, Salem MZ (2016) Isolation of bioactive phenazine-1-carboxamide from the soil bacterium Pantoeaagglomerans and study of its anticancer potency on different cancer cell lines. J AOAC Int 99(5):1233–1239. https://doi.org/10.5740/jaoacint.16-0090

Anantha PS, Deventhiran M, Saravanan P, Anand D, Rajarajan S (2016) A comparative GC-MS analysis of bacterial secondary metabolites of Pseudomonas species. Pharma Innov J 5(4):84–89

Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, Soerjomataram I (2022) Current and future burden of breast cancer: global statistics for 2020 and 2040. Breast 66:15–23. https://doi.org/10.1016/j.breast.2022.08.010

Ashfaq M, Afzal A, Javed MA, Ali M, Rasool B, Shaheen S, Rasheed A (2021) Identification and pathogenic characterization of bacteria causing rice grain discoloration in Pakistan. Int J Biol Biotechnol 18:239–346

Ayan C, Uttam KR, Dinesh H (2017) Protein active site structure prediction strategy and algorithm. Int J Curr Eng Technol 7:1092–1096. https://doi.org/10.14741/Ijcet/22774106/7.3.2017.53

Ayuningrum D, Liu Y, Riyanti, Sibero MT, Kristiana R, Asagabaldan MA, Schaeberle TF (2019) Tunicate-associated bacteria show a great potential for the discovery of antimicrobial compounds. PLoS One 14(3):e0213797. https://doi.org/10.1371/journal.pone.0213797

Azizah MR, Hanum HA, Norahmawati E, Fakurazi S, Kawamoto Y, Permana S, Endharti AT (2024) Systematic review on the efficacy and safety of ixabepilone-based chemotherapy regimen in triple-negative breast cancer. J Pharm Pharmacogn Res 12(4):722–734. https://doi.org/10.56499/jppres23.1869_12.4.722

Balogun TA, Iqbal MN, Saibu OA, Akintubosun MO, Lateef OM, Nneka UC, Omoboyowa DA (2022) Discovery of potential HER2 inhibitors from Mangifera indica for the treatment of HER2-positive breast cancer: an integrated computational approach. J Biomol Struct Dyn 40(23):12772–12784. https://doi.org/10.1080/07391102.2021.1975570

Behzadi R, Hormati A, Eivaziatashbeik K, Ahmadpour S, Khodadust F, Zaboli F, Seidi K (2021) Evaluation of anti-tumor potential of Lactobacillus acidophilus ATCC4356 culture supernatants in MCF-7 breast cancer. Anti-Cancer Agents Med Chem 21(14):1861–1870. https://doi.org/10.2174/1871520621666201207085239

Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kauserud H (2010) ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC Microbiol 10:1–9. https://doi.org/10.1186/1471-2180-10-189

Burianek LL, Yousef AE (2000) Solvent extraction of bacteriocins from liquid cultures. Lett Appl Microbiol 31(3):193–197. https://doi.org/10.1046/j.1365-2672.2000.00802.x

Burke MJ, Ziegler DS, Bautista F, Attarbaschi A, Gore L, Locatelli F, Baruchel A (2022) Phase 1b study of carfilzomib with induction chemotherapy in pediatric relapsed/refractory acute lymphoblastic leukemia. Pediatr Blood Cancer 69(12):e29999. https://doi.org/10.1002/pbc.29999

Burley SK, Bhikadiya C, Bi C, Bittrich S, Chen L, Crichlow GV et al (2021) RCSB Protein Data Bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering, and energy sciences. Nucleic Acids Res 49:D437–D451. https://doi.org/10.1093/nar/gkaa1038

Caicedo NH, Kumirska J, Neumann J, Stolte S, Thöming J (2012) Detection of bioactive exometabolites produced by the filamentous marine cyanobacterium Geitlerinema sp. Mar Biotechnol 14:436–445. https://doi.org/10.1007/s10126-011-9424-1

Carradori S, Guglielmi P, Luisi G, Secci D (2022) Nitrogen-and sulfur-containing heterocycles as dual antioxidant and anticancer agents. In handbook of oxidative stress in cancer: Mechanistic Aspects. Springer Nature Singapore, pp. 2571–2588.https://doi.org/10.1007/978-981-15-9411-3_180

Caulier S, Nannan C, Gillis A, Licciardi F, Bragard C, Mahillon J (2019) Overview of the antimicrobial compounds produced by members of the Bacillus subtilis group. Front Microbiol 10:302. https://doi.org/10.3389/fmicb.2019.00302

Chalasani AG, Dhanarajan G, Nema S, Sen R, Roy U (2015) An antimicrobial metabolite from Bacillus sp: significant activity against pathogenic bacteria including multidrug-resistant clinical strains. Front Microbiol 6:1335. https://doi.org/10.3389/fmicb.2015.01335

Chen H, Cui J, Wang P, Wang X, Wen J (2020) Enhancement of bleomycin production in Streptomyces verticillus through global metabolic regulation of N-acetylglucosamine and assisted metabolic profiling analysis. Microb Cell Fact 19:1–17. https://doi.org/10.1186/s12934-020-01301-8

Cousins KR (2011) Computer review of ChemDraw Ultra 12.0. J Am Chem Soc 133(21):8388. https://doi.org/10.1021/ja204075s

Cristofanilli M (2012) Advancements in the treatment of metastatic breast cancer (MBC): the role of ixabepilone. J Oncol 2012(1):703858. https://doi.org/10.1155/2012/703858

Daina A, Michielin O, Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of small molecules. Sci Rep 7:42717. https://doi.org/10.1038/srep42717

Dan AK, Manna A, Ghosh S, Sikdar S, Sahu R, Parhi PK, Parida S (2021) Molecular mechanisms of the lipopeptides from Bacillus subtilis in the apoptosis of cancer cells - a review on its current status in different cancer cell lines. Adv Cancer Biol Metastasis 3:10001. https://doi.org/10.1016/j.adcanc.2021.100019

Drakontaeidi A, Papanotas I, Pontiki E (2024) Multitarget pharmacology of sulfur–nitrogen heterocycles: anticancer and antioxidant perspectives. Antioxidants 13(8):898. https://doi.org/10.3390/antiox13080898

Elmanama IA, Elmanama AA, Al Zaharna MM, Al-Reefi MR (2020) In vitro anticancer activity effect of extracellular metabolites of some bacterial species on HeLa cell line. IUG J Nat Stud 28(2) https://doi.org/10.33976//IUGNS.28.2/2020/2

En-Nahli F, Baammi S, Hajji H, Alaqarbeh M, Lakhlifi T, Bouachrine M (2023) High-throughput virtual screening approach of natural compounds as target inhibitors of plasmepsin-II. J Biomol Struct Dyn 41(19):10070–10080. https://doi.org/10.1080/07391102.2022.2152871

Faruk M (2021) Breast cancer resistance to chemotherapy: When should we suspect it and how can we prevent it? Ann Med Surg 70:102793. https://doi.org/10.1016/j.amsu.2021.102793

Franzoi MA, Agostinetto E, Perachino M, Del Mastro L, de Azambuja E, Vaz-Luis I, Lambertini M (2021) Evidence-based approaches for the management of side-effects of adjuvant endocrine therapy in patients with breast cancer. Lancet Oncol 22(7):e303–e313. https://doi.org/10.1016/S1470-2045(20)30666-5

Gam LH (2012) Breast cancer and protein biomarkers. World J Exp Med 2(5):86–91. https://doi.org/10.5493/wjem.v2.i5.86

Gay F, Günther A, Offidani M, Engelhardt M, Salvini M, Montefusco V, Gramatzki M (2021) Carfilzomib, bendamustine, and dexamethasone in patients with advanced multiple myeloma: the EMN09 phase 1/2 study of the european myeloma network. Cancer 127(18):3413–3421. https://doi.org/10.1002/cncr.33647

Gil-Turnes MS, Hay ME, Fenical W (1989) Symbiotic marine bacteria chemically defend crustacean embryos from a pathogenic fungus. Science 246(4926):116–118. https://doi.org/10.1126/science.2781297

Gislin D, Sudarsanam D, Raj GA, Baskar K (2018) Antibacterial activity of soil bacteria isolated from Kochi, India and their molecular identification. J Genet Eng Biotechnol 16(2):287–294. https://doi.org/10.1016/j.jgeb.2018.05.010

Grafe U, Radics L (1986) Isolation and structure elucidation of 6-(3'-methylbuten-2'-yl) isatin, an unusual metabolite from Streptomyces albus. J Antibiot 39(1):162–163. https://doi.org/10.7164/antibiotics.39.162

Hamed AA, Ghada Abdel-Razik G, Battah MG, Hassan M (2024) Bioactive metabolites from Streptomyces sp RSE with potential anticancer and antioxidant activity. Egypt J Chem 67(4):115–125. https://doi.org/10.21608/ejchem.2023.226993.8357

Houghton SC, Hankinson SE (2021) Cancer progress and priorities: breast cancer. Cancer Epidemiol Biomarkers Prev 30(5):822–844. https://doi.org/10.1158/1055-9965.EPI-20-1193

Huang B, Kong L, Wang C, Ju F, Zhang Q, Zhu J, Gong T, Zhang H, Yu C, Zheng WM, Bu D (2023) Protein structure prediction: challenges, advances, and the shift of research paradigms. Genomics Proteomics Bioinform 21(5):913–925. https://doi.org/10.1016/j.gpb.2022.11.014

Jeong SY, Ishida K, Ito Y, Okada S, Murakami M (2003) Bacillamide, a novel algicide from the marine bacterium, Bacillus sp. SY-1, against the harmful dinoflagellate Cochlodiniumpolykrikoides. Tetrahedron Lett. 44(43):8005–8007. https://doi.org/10.1016/j.tetlet.2003.08.115

Jeong SY, Park SY, Kim YH, Kim M, Lee SJ (2008) Cytotoxicity and apoptosis induction of Bacillus vallismortis BIT-33 metabolites on colon cancer carcinoma cells. J Appl Microbiol 104(3):796–807. https://doi.org/10.1111/j.1365-2672.2007.03615.x

Kalia VC, Patel SK, Cho BK, Wood TK, Lee JK (2022) Emerging applications of bacteria as antitumor agents. Semin Cancer Biol 86:1014–1025. https://doi.org/10.1016/j.semcancer.2021.05.012

Kannan MN, Sethi S, Badoni A, Chamoli V, Bahuguna NC (2018) Isolation and characterization of bacterial isolates from agriculture field soil of Roorkee region. J Pharmacogn Phytochem 7(5S):108–110

Kaspar F, Neubauer P, Gimpel M (2019) Bioactive secondary metabolites from Bacillus subtilis: a comprehensive review. J Nat Prod 82(7):2038–2053. https://doi.org/10.1021/acs.jnatprod.9b00110

Kennedy RK, Naik PR, Veena V, Lakshmi BS, Lakshmi P, Krishna R, Sakthivel N (2015) 5-Methyl phenazine-1-carboxylic acid: a novel bioactive metabolite by a rhizosphere soil bacterium that exhibits potent antimicrobial and anticancer activities. Chem-Biol Interact. https://doi.org/10.1016/j.cbi.2015.03.002

Kumari NP, Ram MR (2021) Isolation and characterization of anticancerous bioactive compounds from marine Bacillus subtilis. J Adv Sci Res 12(02):203–211. https://doi.org/10.55218/JASR.s2202112228

Kyrgiou M, Salanti G, Pavlidis N, Paraskevaidis E, Ioannidis JP (2006) Survival benefits with diverse chemotherapy regimens for ovarian cancer: meta-analysis of multiple treatments. J Natl Cancer Inst 98(22):1655–1663. https://doi.org/10.1093/jnci/djj443

Laliani G, Sorboni SG, Lari R, Yaghoubi A, Soleimanpour S, Khazaei M, Avan A (2020) Bacteria and cancer: Different sides of the same coin. Life Sci 246:117398. https://doi.org/10.1016/j.lfs.2020.117398

Laursen JB, Nielsen J (2004) Phenazine natural products: biosynthesis, synthetic analogues, and biological activity. Chem Rev 104(3):1663–1686. https://doi.org/10.1021/cr020473j

Li J, Ren J, Sun W (2017) Systematic review of ixabepilone for treating metastatic breast cancer. Breast Cancer 24:171–179. https://doi.org/10.1007/s12282-016-0717-0

Liu M, Jia Y, Xie Y, Zhang C, Ma J, Sun C, Ju J (2019) Identification of the actinomycin D biosynthetic pathway from marine-derived Streptomyces costaricanus SCSIO ZS0073. Mar Drugs 17(4):240. https://doi.org/10.3390/md17040240

Liu Y, Lau HCH, Yu J (2023) Microbial metabolites in colorectal tumorigenesis and cancer therapy. Gut Microbes 15(1):2203968. https://doi.org/10.1080/19490976.2023.2203968

Marcelo P, Gontier E, Dauwe R (2019) Metabolic markers for the yield of lipophilic indole alkaloids in dried woad leaves (Isatis tinctoria L.). Phytochemistry 163:89–98. https://doi.org/10.1016/j.phytochem.2019.04.006

McCoy CS, Mannion AJ, Feng Y, Madden CM, Artim SC, Au GG, Fox JG (2021) Cytotoxic Escherichia coli strains encoding colibactin, cytotoxic necrotizing factor, and cytolethal distending toxin colonize laboratory common marmosets (Callithrix jacchus). Sci Rep 11(1):2309. https://doi.org/10.1038/s41598-020-80000-1

Mishra APSS (2013) Isolation and biochemical characterization of antibiotic-producing microorganism from waste soil samples of certain industrial areas of India. https://doi.org/10.9790/3008-0568089

Mitri Z, Constantine T, O'Regan R (2012) The HER2 receptor in breast cancer: pathophysiology, clinical use, and new advances in therapy. Chemother Res Pract 2012(1):743193. https://doi.org/10.1155/2012/743193

Mohan CD, Rangappa S, Nayak SC, Jadimurthy R, Wang L, Sethi G, Rangappa KS (2022) Bacteria as a treasure house of secondary metabolites with anticancer potential. Semin Cancer Biol 86:998–1013. https://doi.org/10.1016/j.semcancer.2021.05.006

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65(1–2):55–63. https://doi.org/10.1016/0022-1759(83)90303-4

Nakkarach A, Foo HL, Song AAL, Mutalib NEA, Nitisinprasert S, Withayagiat U (2021) Anti-cancer and anti-inflammatory effects elicited by short chain fatty acids produced by Escherichia coli isolated from healthy human gut microbiota. Microb Cell Fact 20:1–17. https://doi.org/10.1186/s12934-020-01477-z

Nascimento Chaves KR, França MLT, Mendes ALO, Cardoso PP, Vasconcelos KBP, Santana RCF, Silva SKR (2024) Antibacterial, antioxidant and anticancer activities of the Streptomyces PML5 strain isolated from carbonate rocks in the Amazon. https://doi.org/10.21203/rs.3.rs-4069286/v1

Numan M, Shah M, Asaf S, Ur Rehman N, Al-Harrasi A (2022) Bioactive compounds from endophytic bacteria Bacillus subtilis strain EP1 with their antibacterial activities. Metabolites 12(12):1228. https://doi.org/10.3390/metabo12121228

Ongena M, Jacques P (2008) Bacillus lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol 16(3):115–125. https://doi.org/10.1016/j.tim.2007.12.009

Osama N, Bakeer W, Raslan M, Soliman HA, Abdelmohsen UR, Sebak M (2022) Anti-cancer and antimicrobial potential of five soil Streptomycetes: A metabolomics-based study. R Soc Open Sci 9(2):211509. https://doi.org/10.1098/rsos.211509

Partridge AH, Winer EP (2004) Long-term complications of adjuvant chemotherapy for early-stage breast cancer. Breast Dis 21(1):55–64. https://doi.org/10.3233/BD-2004-21108

Pereira DM, Valentão P, Pereira JA, Andrade PB (2009) Phenolics: from chemistry to biology. Molecules 14(6):2202–2211. https://doi.org/10.3390/molecules14062202

Raghava Rao KV, Mani P, Satyanarayana B, Raghava Rao T (2017) Purification and structural elucidation of three bioactive compounds isolated from Streptomyces coelicoflavus BC 01 and their biological activity. Biotech 7:1–12. https://doi.org/10.1007/s13205-016-0581-9

Rahman H, Khan I, Hussain A, Shahat AA, Tawab A, Qasim M, Khan SN (2018) Glycyrrhiza glabra HPLC fractions: identification of AldehydoIsoophiopogonone and Liquirtigenin having activity against multidrug resistant bacteria. BMC Complement Altern Med 18:1–6. https://doi.org/10.1186/s12906-018-2207-8

Rajivgandhi G, Muneeswaran T, Maruthupandy M, Ramakritinan CM, Saravanan K, Ravikumar V, Manoharan N (2018) Antibacterial and anticancer potential of marine endophytic actinomycetes Streptomyces coeruleorubidus GRG 4 (KY457708) compound against colistin-resistant uropathogens and A549 lung cancer cells. Microb Pathog 125:325–335. https://doi.org/10.1016/j.micpath.2018.09.025

Rasheed MA, Iqbal MN, Saddick S, Ali I, Khan FS, Kanwal S, Awais M (2021) Identification of lead compounds against Scm (fms10) in Enterococcus faecium using computer-aided drug designing. Life 11(2):77. https://doi.org/10.3390/life11020077

Riaz A, Kaleem A, Abdullah R, Iqtedar M, Hoessli DC, Aftab M (2024) In silico approaches to study the human asparagine synthetase: An insight of the interaction between the enzyme active sites and its substrates. PLoS ONE 19(8):e0307448. https://doi.org/10.1371/journal.pone.0307448

Ritchie TJ, Ertl P, Lewis R (2011) The graphical representation of ADME-related molecule properties for medicinal chemists. Drug Discov Today 16(1–2):65–72. https://doi.org/10.1016/j.drudis.2010.11.002

Sait KHW, Mashraqi M, Khogeer AA, Alzahrani O, Anfinan NM, Sait HK, Almutairi A, Alam Q (2020) Molecular docking analysis of HER-2 inhibitor from the ZINC database as anticancer agents. Bioinformation 16(11):882–887. https://doi.org/10.6026/97320630016882

Salazar B, Ortiz A, Keswani C, Minkina T, Mandzhieva S, Pratap Singh S, Sansinenea E (2023) Bacillus spp. as bio-factories for antifungal secondary metabolites: innovation beyond whole organism formulations. Microb Ecol 86(1):1–24. https://doi.org/10.1007/s00248-022-02044-2

San-Diego CA (2021) USA, BIOVIA; Dassault Systèmes. Discovery Studio Visualizer. Dassault Systèmes, v21.1.0.20298.

Schettini F, Pascual T, Conte B, Chic N, Brasó-Maristany F, Galván P, Prat A (2020) HER2-enriched subtype and pathological complete response in HER2-positive breast cancer: a systematic review and meta-analysis. Cancer Treat Rev 84:101965. https://doi.org/10.1016/j.ctrv.2020.101965

Schrödinger L, DeLano W., 2020. PyMOL [Internet]. Available from: http://www.pymol.org/pymol

Sebola TE, Uche-Okereafor NC, Mekuto L, Makatini MM, Green E (2020). Mavumengwana. https://doi.org/10.1155/2020/8839490

Sereena MC, Sebastian D (2020) Evaluation of anticancer and antihemolytic activity of azurin, a novel bacterial protein from Pseudomonas aeruginosa SSj. Int J Pept Res Ther 26(1):459–466. https://doi.org/10.1007/s10989-019-09851-1

Sethi Y, Vora V, Anyagwa OE, Turabi N, Abdelwahab M, Kaiwan O, Padda I (2024) Streptomyces paradigm in anticancer therapy: a state-of-the-art review. Curr Cancer Ther Rev 20(4):386–401. https://doi.org/10.2174/0115733947254550230920170230

Shaaban KA, Shaaban M, Nair V, Schuhmann I, Win HY, Lei L, Laatsch H (2016) Structure elucidation and synthesis of hydroxylated isatins from Streptomycetes. Z Naturforsch B 71(12):1191–1198. https://doi.org/10.1515/znb-2016-0143

Shaikh S, Younis M, Yingying S, Tanziela T, Yuan L (2023) Bleomycin loaded exosomes enhanced antitumor therapeutic efficacy and reduced toxicity. Life Sci 330:121977. https://doi.org/10.1016/j.lfs.2023.121977

Shalini TS, Manivel G, Ragothaman P, Velu RK, Senthilraja P (2024) Secondary metabolite profiling using HR-LCMS, antioxidant and anticancer activity of Bacillus cereus PSMS6 methanolic extract: In silico and in vitro study. Biotechnol Rep 42:e00842

Shamsee ZR, Al-Saffar AZ, Al-Shanon AF, Al-Obaidi JR (2019) Cytotoxic and cell cycle arrest induction of pentacyclic triterpenoids separated from Lantana camara leaves against MCF-7 cell line in vitro. Mol Biol Rep 46:381–390. https://doi.org/10.1007/s11033-018-4482-3

Shao Y, Wang XY, Qiu X, Niu LL, Ma ZL (2021) Isolation and purification of a new Bacillus subtilis strain from deer dung with antimicrobial and anti-cancer activities. Curr Med Sci 41:832–840. https://doi.org/10.1007/s11596-021-2383-5

Sihem BM, Rafik E, Mathieu F, Mohamed C, Lebrihi A (2011) Identification and partial characterization of antifungal and antibacterial activities of two Bacillus sp. strains isolated from salt soil in Tunisia. Afr J Microbiol Res 5(13):1599. https://doi.org/10.5897/AJMR11.073

Socha AM, Long RA, Rowley DC (2007) Bacillamides from a hypersaline microbial mat bacterium. J Nat Prod 70(11):1793–1795. https://doi.org/10.1021/np070126a

Speranza J, Miceli N, Taviano MF, Ragusa S, Kwiecień I, Szopa A, Ekiert H (2020) Isatis tinctoria L. (Woad): A review of its botany, ethnobotanical uses, phytochemistry, biological activities, and biotechnological studies. Plants 9(3):298. https://doi.org/10.3390/plants9030298

Subbiah U, Dinakarkumar Y, Jeyaraman M (2024) Spongia officinalis-associated Pseudomonas fluorescens as a reservoir of bioactive compounds: A novel source of natural anticancer compounds. J Trop Biodivers Biotechnol 9(3):90693. https://doi.org/10.22146/jtbb.90693

Terefe EM, Ghosh A (2022) Molecular docking, validation, dynamics simulations, and pharmacokinetic prediction of phytochemicals isolated from Croton dichogamus against the HIV-1 reverse transcriptase. Bioinform Biol Insights 16:11779322221125604. https://doi.org/10.1177/11779322221125605

Thomas AT, Rao JV, Subrahmanyam VM, Chandrashekhar HR, Maliyakkal N, Kisan TK, Joseph A, Udupa N (2011) In vitro anticancer activity of microbial isolates from diverse habitats. Braz J Pharm Sci 47(2):279–287. https://doi.org/10.1590/S1984-82502011000200009

Thorn CF, Oshiro C, Marsh S, Hernandez-Boussard T, McLeod H, Klein TE, Altman RB (2011) Doxorubicin pathways: pharmacodynamics and adverse effects. Pharmacogenet Genomics 21(7):440–446. https://doi.org/10.1097/FPC.0b013e32833ffb56

Tran C, Cock IE, Chen X, Feng Y (2022) Antimicrobial Bacillus: metabolites and their mode of action. Antibiotics 11(1):88. https://doi.org/10.3390/antibiotics11010088

Trayes KP, Cokenakes SE (2021) Breast cancer treatment. Am Fam Phys 104(2):171–178

Triningsih DW, Harunari E, Fukaya K, Oku N, Urabe D, Igarashi Y (2023) Species-specific secondary metabolism by actinomycetes of the genus Phytohabitans and discovery of new pyranonaphthoquinones and isatin derivatives. J Antibiot 76(5):249–259. https://doi.org/10.1038/s41429-023-00605-2

Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 3:455–461. https://doi.org/10.1002/jcc.21334

Tyc O, Song C, Dickschat JS, Vos M, Garbeva P (2017) The ecological role of volatile and soluble secondary metabolites produced by soil bacteria. Trends Microbiol 25(4):280–292. https://doi.org/10.1016/j.tim.2016.12.002

Uddin MN, Chowdhury MH (2022) Enumeration, isolation, and characterization of bacteria of Gopalpur soil series of Bangladesh. Interdiscip J Appl Basic Subj 2(2):21–26. https://doi.org/10.0002/ijabs-22b-06001

Wang Y, Wang Y, Sun T, Xu J (2024) Bacteriocins in cancer treatment: mechanisms and clinical potentials. Biomolecules 14(7):831. https://doi.org/10.3390/biom14070831

White TJ, Bruns T, Lee SJWT, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc Guide Methods Appl 18(1):315–322

Wu C, Du C, Gubbens J, Choi YH, Van Wezel GP (2015) Metabolomics-driven discovery of a prenylated isatin antibiotic produced by Streptomyces species MBT28. J Nat Prod 78(10):2355–2363. https://doi.org/10.1021/acs.jnatprod.5b00276

Yaghoubi A, Ghazvini K, Khazaei M, Hasanian SM, Avan A, Soleimanpour S (2022) The use of Clostridium in cancer therapy: a promising way. Rev Res Med Microbiol 33(2):121–127. https://doi.org/10.1097/MRM.0000000000000281

Zhang KP, Fang X, Zhang Y, Chao M (2021) The prognosis of cancer patients undergoing liposomal doxorubicin-based chemotherapy: a systematic review and meta-analysis. Medicine 100(34):e26690. https://doi.org/10.1097/MD.0000000000026690

Zhang S, Croppi G, Hu H, Li Y, Zhu C, Wu F, Li Z (2022) Bacillamide F, extracted from marine Bacillus atrophaeus C89, preliminary effects on leukemia cell lines. Biology 11(12):1712. https://doi.org/10.3390/biology11121712

Zhang S, Jin Z, Bao L, Shu P (2024) The global burden of breast cancer in women from 1990 to 2030: assessment and projection based on the global burden of disease study 2019. Front. Oncol 14:1364397. https://doi.org/10.3389/fonc.2024.1364397