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Research Article

Deciphering the anti-COVID-19 compounds from *Streptomyces* isolated from the tea rhizosphere of Darjeeling Hills, India

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Abstract

The outbreak of the novel coronavirus SARS-CoV-2, originating in China, has escalated into a global pandemic, causing severe health and socioeconomic impacts. Despite the development of vaccines, emerging variants continue to pose significant threats to human health. Therefore, the search for novel antiviral agents from natural sources remains a global priority. Streptomyces, a prolific producer of bioactive secondary metabolites, offers a promising avenue for discovering anti-COVID-19 compounds. Streptomyces strains were isolated from the tea rhizosphere soil of the Darjeeling hills, India. The bioactive metabolites produced by the selected strain were identified using Gas Chromatography–Mass Spectrometry (GC–MS). The anti-COVID-19 potential of these compounds was evaluated through *in silico* molecular docking and molecular dynamics (MD) simulation studies targeting the SARS-CoV-2 main protease (Mpro). Among the detected compounds, Vinylbital, Ergotaman, and Bis(2-ethylhexyl) phthalate exhibited strong binding affinities toward the active site of Mpro, suggesting their potential inhibitory effects. The stability of these ligand–protein complexes was further supported by RMSD and RMSF analyses during MD simulations, validating the reliability of the docking results. This study reports, for the first time, the discovery of *Streptomyces*-derived bioactive compounds from the tea rhizosphere of Darjeeling hills with promising *in silico* anti-COVID-19 activity. These findings highlight the potential of rhizospheric actinobacteria as a valuable source of antiviral metabolites and provide a foundation for future experimental validation and drug development efforts.

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Introduction

The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to impose enormous burdens globally. First identified in Wuhan, Hubei Province, China, in December 2019, SARS-CoV-2 rapidly spread and was declared a pandemic by the World Health Organization in March 2020 (Abel et al., 2020). The disease has resulted in extremely high morbidity and mortality, overwhelmed healthcare infrastructure in many regions, and produced both acute disease and long-term sequelae ("Long COVID") in survivors (Adeyemo et al., 2020). Given the continuing waves driven by emerging variants and observed warning of vaccine-induced immunity over time, there remains an urgent need for effective treatments and novel antiviral agents (Aftab et al., 2020).

SARS-CoV-2 is an enveloped, positive-sense RNA virus with four main structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N) (Ahsan et al.,2017). Cell entry is mediated via binding of the receptor-binding domain (RBD) of the S protein to the human angiotensin-converting enzyme 2 (ACE2) receptor, followed by activation by proteases such as TMPRSS2 or cathepsins (Alamri et al., 2020; Al-Khafaji et al.,2021). Once inside, viral replication requires the action of non-structural proteins, including the main protease

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(Mpro, also called 3CLpro or nsp5), the RNA-dependent RNA polymerase (RdRp, nsp12), helicase (nsp13), and others. These proteins are critical to processing viral polyproteins and forming the replicase complex (Amin et al., 2021).

Currently, some antiviral treatments have been approved or granted emergency use authorization. For example, Paxlovid (nirmatrelvir + ritonavir), a main protease inhibitor, has shown substantial efficacy in reducing hospitalizations and deaths when administered early to high-risk patients (Balachandran et al., 2012). Remdesivir, an RdRp inhibitor, also reduces disease duration in severe cases (Balachandran et al., 2012). Other protease inhibitors (e.g., lopinavir/ritonavir) initially showed promise but have largely underperformed in trials (Baskaran et al.,2015). Monoclonal antibodies and host-directed antivirals (e.g., TMPRSS2 inhibitors, cathepsin inhibitors) have been explored, yet many have reduced effectiveness against newer variants (Baskaran et al.,2015; Chen et al., 2020). Further complicating treatment is the emergence of antiviral-resistant SARS-CoV-2 strains, especially in immunocompromised individuals; mutations in Mpro (nsp5) and RdRp (nsp12) have been documented that confer reduced sensitivity to agents like Paxlovid and remdesivir (Corre et al., 1990).

Vaccination remains the most effective strategy for preventing severe disease, hospitalization, and death (Dai et al., 2020). Nonetheless, vaccine effectiveness varies with variant, and booster dosing, vaccine redesign, and hybrid immunity effects are still being characterized (Coccolini et al., 2026). These considerations highlight the importance of complementing vaccination with therapeutics, especially for populations at risk, breakthrough infections, or those who cannot mount sufficient vaccine responses (Dharan and Prasad, 2018). In addition, post-COVID complications (cardiopulmonary, neurological, etc.) illustrate that prevention alone is not sufficient; therapeutics to reduce viral replication and ameliorate inflammatory damage are essential (Dholakiya et al., 2017).

Natural products continue to be a rich source of drug leads. Phytochemicals, small molecules from plants, and chemical compounds have been widely studied via *in silico*, *in vitro*, and some *in vivo* models for potential anti-SARS-CoV-2 activity (El-Mehalawy et al., 2005). However, comparatively fewer studies have explored *bacterial* (especially actinobacterial) secondary metabolites, particularly those derived from soil environments, as possible therapeutics for COVID-19. This is in spite of the fact that bacterial natural products have a long history in antibiotics, anticancer, antiviral, and other medicinal uses (El-Naggar et al., 2017).

The genus *Streptomyces*, belonging to the phylum Actinobacteria, is especially prolific in producing structurally diverse secondary metabolites, many with antibacterial, antiviral, antifungal, anticancer, immunomodulatory, or other bioactive properties (Gordon et al.,2020). Recent investigations have begun to uncover *Streptomyces* compounds with antiviral activity: for example, *Streptomyces* sp. strain 196 has yielded natural products (e.g., K-252C and daunorubicin) that in silico show favorable binding to SARS-CoV-2 holoRdRp and nsp13, suggesting potential to inhibit viral replication (Goyal and Goyal,2020). Another study identified pseudo-tetrapeptides ("omicsynins") from *Streptomyces* sp. 1647 that are active against influenza A virus and human coronavirus HCoV-229E (Jaiswal et al.,2020).Bafilomycins derived from *Streptomyces* sp. HTL16 (isolated from feces) showed potent antiviral activity against influenza A and SARS-CoV-2 in vitro via inhibition of endosomal ATP-driven proton pumps (Johnson et al., 2008). Also, an in silico screening of compounds from *Streptomyces ambofaciens* identified "Stambomycin B" as a candidate with strong binding affinity against multiple SARS-CoV-2 variants' RBDs (e.g., Alpha, Beta, Delta, Omicron sublineages), suggesting potential pan-variant viral entry inhibition (Jia and Gong, 2019). These findings reinforce the promise of *Streptomyces*-derived molecules for antiviral drug discovery in the COVID-19 context.

In this light, our present study focuses on a *Streptomyces* strain (designated *Streptomyces* sp. SDr-06) isolated from the tea rhizosphere soils of Darjeeling, India. Tea rhizospheres represent a micro-ecological niche rich in interactions, secondary metabolite diversity, and potentially untapped actinobacterial diversity. We aimed to explore whether secondary metabolites produced by *Streptomyces* sp. SDr-06 has structural and functional properties that could make it a candidate for anti-SARS-CoV-2 therapy. Specifically, we employed metabolite profiling (e.g., GC-MS), followed by *in silico* methods including molecular docking and molecular dynamics simulations targeting SARS-CoV-2 proteins central to viral entry and replication (e.g., Mpro, RdRp, RBD-ACE2 interface). We hypothesize that these compounds may show binding affinities and stability comparable to or better than known antiviral agents,

thereby contributing novel lead molecules for anti-COVID-19 drug development.

2. Materials and methods

2.1. Soil Sampling

Soil samples were collected from the Dhajea Tea Garden, located in Darjeeling, West Bengal, India (26.9079° N, 88.2230° E). Samples were aseptically collected in sterile plastic containers, tightly sealed, and transported to the laboratory for processing. The soil was air-dried at room temperature (25 ± 2°C) for 3–4 days and stored under dry conditions until further use for the isolation of actinobacteria.

2.2. Chemicals and Reagents

All chemicals, media, and reagents used in this study were of analytical grade and procured from Merck (Mumbai, India), Hi-Media Laboratories Pvt. Ltd. (Mumbai, India), and Sigma-Aldrich (USA).

2.3. Isolation of Actinobacteria

One gram of dried soil was suspended in 9 mL of sterile saline solution (0.85% NaCl) and serially diluted up to 10^{-6}. Aliquots (0.1 mL) from each dilution were plated on Inorganic Salts Starch Agar (ISSA) medium (Taddei et al., 2006) using the spread plate method. The medium was supplemented with rifampicin (5 µg/mL) and fluconazole (25 µg/mL) to minimize bacterial and fungal contamination (Dholakiya et al.,2017; Rao and Rao,2013). Plates were incubated at 30°C for seven days. Distinct colonies with actinobacterial morphology were sub-cultured onto ISP4 agar medium (Rashad et al., 2015) and purified through successive streaking. The purified isolates were maintained on ISP4 slants, incubated at 30°C for seven days, and preserved at 4°C for further studies (Shepherd et al.,2010).

2.4. Genomic DNA Extraction and 16S rDNA Sequencing

Genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method (William et al.,2012). Cultures were grown in Bennett's broth, centrifuged at 8000 × g for 10 min at 4°C, and the resulting pellet was washed with 1× TE buffer. The cells were treated with lysozyme (10 mg/mL) for 5 min, followed by the addition of 10% SDS and proteinase K (20 mg/mL), and incubated at 37°C for 1 h. Subsequently, CTAB/NaCl solution was added, and the mixture was incubated at 65°C for 10 min. DNA was extracted using chloroform: isoamyl alcohol (24:1), centrifuged (8000 × g, 20 min), and precipitated with 0.6 volumes of chilled isopropanol at −20°C overnight. The DNA pellet was washed with 70% ethanol, air-dried, and resuspended in 1× TE buffer for storage at 4°C.

The 16S rDNA region was amplified by PCR using actino-specific primers ACT235F (5′-CGC GGC CTA TCA GCT TGT TG-3′) and ACT878R (5′-CCG TAC TCC CCA GGC GGG G-3′) (Stach et al., 2003). PCR reactions (25 µL) contained 12.5 µL GoTaq Green Master Mix (Promega, USA), 1.25 µL each of forward and reverse primers, 2 µL template DNA, and 8 µL nuclease-free water. The amplification program included initial denaturation at 95°C (4 min), followed by 40 cycles of denaturation (95°C, 45 s), annealing (72°C, 60 s), and extension (72°C, 60 s), with a final extension at 72°C (5 min). The amplified 16S rDNA fragments were sequenced, and species identification was performed via BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) against the NCBI database (Johnson et al., 2008).

2.5. Cultivation of Streptomyces sp. SDr-06 and Extraction of Bioactive Compounds

The purified isolate *Streptomyces* sp. SDr-06 was cultivated on ISP4 plates at 30°C for seven days. A loopful of mature spores was inoculated into a sterilized fermentation medium (peptone 10 g, NaCl 10 g, glucose 10 g, $CaCO_3$ 1 g, distilled water 1000 mL; pH 7.0) and incubated at 30°C under shaking conditions (180 rpm) for seven days. After fermentation, the culture broth was centrifuged, and the supernatant was stored at −4°C (Ahsan et al., 2017; Singh et al., 2014).

Bioactive metabolites were extracted from the culture filtrate using chloroform in a 1:1 (v/v) ratio. The mixture was agitated vigorously and incubated overnight on a rotary shaker. The organic (chloroform) phase was separated, concentrated by evaporation at 50°C, and pooled to obtain the crude extract (Ahsan et al.,2017; Ravi and Kannabiran, 2018).

2.6. GC–MS Analysis

Gas chromatography–mass spectrometry (GC–MS) analysis of the crude extract was conducted using a Shimadzu GCMS-QP2010 Plus system (Kyoto, Japan) equipped with an AOC-20s headspace sampler and AOC-20i injector. Separation was achieved on an Rtx-5 MS capillary column (30 m × 0.25 mm × 0.25 µm). The injector temperature was set at 260°C (split ratio 10:1). The temperature program was 60°C (3 min), ramped to 300°C at 15°C/min, and held for 18 min. Helium (≥99.999%) was used as the carrier gas at a linear flow of 40.1 cm/s. The ion source and interface temperatures

were maintained at 230°C and 270°C, respectively. The sample (1.0 µL) was injected, and compounds were identified by comparing their mass spectra and retention indices with entries in the WILEY8.LIB, NIST14.LIB, NIST14s.LIB, and Drug Library (PMW_TOX2.LIB) databases.

2.7. Physicochemical Property Analysis of Ligands

Physicochemical and pharmacokinetic properties (ADMET: absorption, distribution, metabolism, excretion, and toxicity) of GC-MS-identified compounds were predicted using SwissADME (http://www.swissadme.ch/). Compounds were also evaluated based on Lipinski's Rule of Five (https://en.wikipedia.org/wiki/Lipinski%27s_rule_of_five). Molecules showing favorable ADMET characteristics and no predicted toxicity were selected as ligands. Their 3D structures were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and converted to pdbqt format using AutoDock Vina (http://vina.scripps.edu/), with torsion angles optimized before docking.

2.8. *Protein Target Preparation*

The main protease (Mpro) of SARS-CoV-2, a crucial enzyme for viral replication, was chosen as the target protein. Its 3D crystal structure (PDB ID: 6LU7) was retrieved from the Protein Data Bank (https://www.rcsb.org/). Water molecules were removed, polar hydrogens were added, and Gasteiger charges were assigned. The processed structure was saved in pdbqt format for docking simulations.

2.9. Molecular Docking Analysis

Molecular docking was performed using AutoDock Vina. Both blind and site-specific docking approaches were applied. Blind docking was used to identify potential binding sites, followed by targeted docking around the highest-affinity pocket. Ligand–protein complexes were ranked by binding affinity (kcal/mol), and the best-scoring interactions were visualized to interpret molecular interactions and binding conformations.

2.10. Assessment of Synergistic Effects

To investigate possible synergistic interactions among active compounds, ligands with binding affinities ≤ −7.0 kcal/mol were selected. Their combined binding potential was assessed following the methodology described in our previous study (Sarkar and Sen,2020).

2.11. Molecular Dynamics (MD) Simulation

Molecular dynamics simulations were carried out using GROMACS software (Pronk et al.,2013) with the GROMACS96 53a6 force field. Each protein–ligand complex was solvated and energy-minimized for 10,000 steps using the steepest descent algorithm. Simulations were performed at 300 K and 1 bar for 100 ns. Root mean square deviation (RMSD) and root mean square fluctuation (RMSF) analyses were conducted to assess the structural stability of complexes.

2.12. MM–GBSA Binding Free Energy Calculation

Binding free energies (ΔG_bind) were calculated using the g_mmpbsa module integrated with GROMACS. The molecular mechanics–generalized Born surface area (MM–GBSA) approach combines molecular mechanics energy (E_MM), polar solvation energy (G_SGB), and nonpolar solvation energy (G_NP) components (Al-Khafaji et al.,2021; Mittal et al.,2020; Sarma et al.,2020; Vijayakumar et al.,2014). The total binding energy was computed using the following relation:

ΔGbind=EMM+GSGB+GNP\Delta G_{bind} = E_{MM} + G_{SGB} + G_{NP}ΔGbind=EMM +GSGB+GNP$\Delta G_{bind} = \Delta G_{complex} - (\Delta G_{protein} + \Delta G_{ligand})$

3. Results and Discussion

3.1. Isolation and DNA extraction

The slow-growing colony of the isolate SDr-06 was typical of *Streptomyces* and was identified by 16S rDNA sequencing, which helped in genus identification. The 16S ribosomal RNA gene sequence was submitted to NCBI, and the accession number allotted was MK294534.1. The NCBI blast of the 16s rDNA sequence showed the highest similarity percentage of 99.34% with *Streptomyces* sp. The culture was also deposited at the National Center for Microbial Resource (NCMR), Pune, and the accession number allocated to the strain SDr-06 was MCC 4430.

3.2. GC-MS Analysis of the culture filtrate extract

After the completion of fermentation and extraction of the culture filtrate with chloroform, the crude extract recovered was slightly yellow to brownish. On GC-MS analysis of the extract produced by SDr-06, it was found that there were many bioactive compounds (Supplementary figure 1a and 1b) with 16 constituents, known to produce antimicrobial, antifungal and antioxidant activities (Supplementary Table 1). Thus the isolate with the potential to

produce antimicrobial compounds has been further explored for its bioactivity against pathogens that cause various diseases. In this context, the antimicrobial compounds were then assessed by in silico molecular docking approach for anti-Covid activity.

3.3. Synergistic activity of three compounds

The ADMET score and Lipinski's rule of five were assessed for all major compounds obtained from GCMS analysis (Supplementary Table 2). From this analysis, it was evident that all the listed compounds have the potency to become drug molecules. As a result, we considered all the compounds as probable ligands. After molecular docking analysis, compounds with a binding affinity of more than -7.0 kcal/mol were only considered for this analysis. We found Vinylbital, Ergotaman and Bis (2-ethylhexyl) phthalate showed the highest binding affinity with the M^{pro} of Covid-19. The binding affinities were -7.2 kcal/mol, -7.5kcal/mol and -7.3 kcal/mol. These three compounds were further considered for revealing the synergistic effect. Sequential docking of those compounds revealed -7.2 kcal/mol, -7.7 kcal/mol and -7.7kcal/mol for Vinylbital, Ergotaman and Bis (2-ethylhexyl) phthalate respectively (Fig.1a). This increase in binding affinities indicated the synergistic effects of these three compounds against Covid M^{pro}. The protein-ligand complex obtained after the synergistic binding activity was further considered for the molecular simulation study. SARS-Cov2 with a large genome of 30kb, codes only a few proteins. Among those proteins MPro, a cysteine protease mediates the maturation cleavage of the poly-proteins during the replication of the virus. This protease is a homodimer with three different domains (I, II and III) (Abel et al.,2020). The main substrate binding site is present in a cleft present between domains I, II and the protomers. The M^{pro} has been reported to be a potential target since denaturation of this protein will hamper the maturation of itself as well as other poly-proteins. Moreover, M^{pro} is also important during viral entry to host cells. Inhibition of this enzyme would halt the viral entry and subsequent infection (Abel et al.,2020). It has been reported that mutations in 285 and 286 of M^{pro} have caused the hyper-activity of the SARS-CoV2. In our study, we have found that vinbytal bind at both 285 and 286 positions of M^{pro} and Phalate binds with positions 286 and 293. This is interesting since these positions are already reported to be vital for M^{pro} activity. Inhibition of these sites through our considered ligands would definitely have some clinical impacts. Moreover, Ergotman was found to bind at a cleft between domain I, II protomer-b as well as protomer-A. Thus, the binding of all three antibiotics with M^{pro} leads to a change in the structural configuration, which may lead to inhibition of M^{pro} functionality and may ultimately cease the viral entry as well as viral replication should the viral RNA has already entered the cell. The MM-GBSA calculation of each protein-ligand complex and synergistically bound complex were calculated and all of them showed favorable binding energy.

Table 1. MM-GBSA calculation of all protein-ligand complexes.

Compounds	MM-GBSA
6LU7- Vinylbital	-56.64
6LU7- Ergotaman	-61.25
6LU7- Bis (2-ethylhexyl) phthalate	-58.97
6LU7- Vinylbital- Ergotaman- Bis (2-ethylhexyl) phthalate	-67.84

3.4. Root Mean Square Deviation analysis

RMSD values are the well-accepted approach for revealing the deviation of protein stability after the binding of ligands. It compares the configuration of the protein backbone of the original structure with that of the protein-ligand complex. A similar RMSD value of protein and protein-ligand complex indicate less structural deviation suggesting the ligands can be used as a probable drug. In this analysis, the RMSD value of the protein-ligand complex did not deviate significantly from that of the original structure. RMSD for 6LU7 varied approximately from 1.5 to 3.1 and the protein-ligand complex

showed the RMSD of 2.5-3.9 (Fig.1b). This indicated that our ligands are potential candidates for anti-covid agents.

3.5. Root mean square fluctuation analysis

Residues that have experienced major fluctuations during the MD simulation process can be determined by RMSF plot (Khan et al.,2021; Muralidharan et al.,2021). RMSF for C-alpha atoms of each amino acid was measured and plotted against the number of residues. The analysis revealed no major changes in their binding pattern. RMSF analysis designated simultaneous binding of three aforementioned ligands to the M^{pro} raised no major complications in terms of protein flexibility and structural conformations (Fig.1c). This reinforces the idea of combined drug therapy with the mentioned ligands against COVID-19.

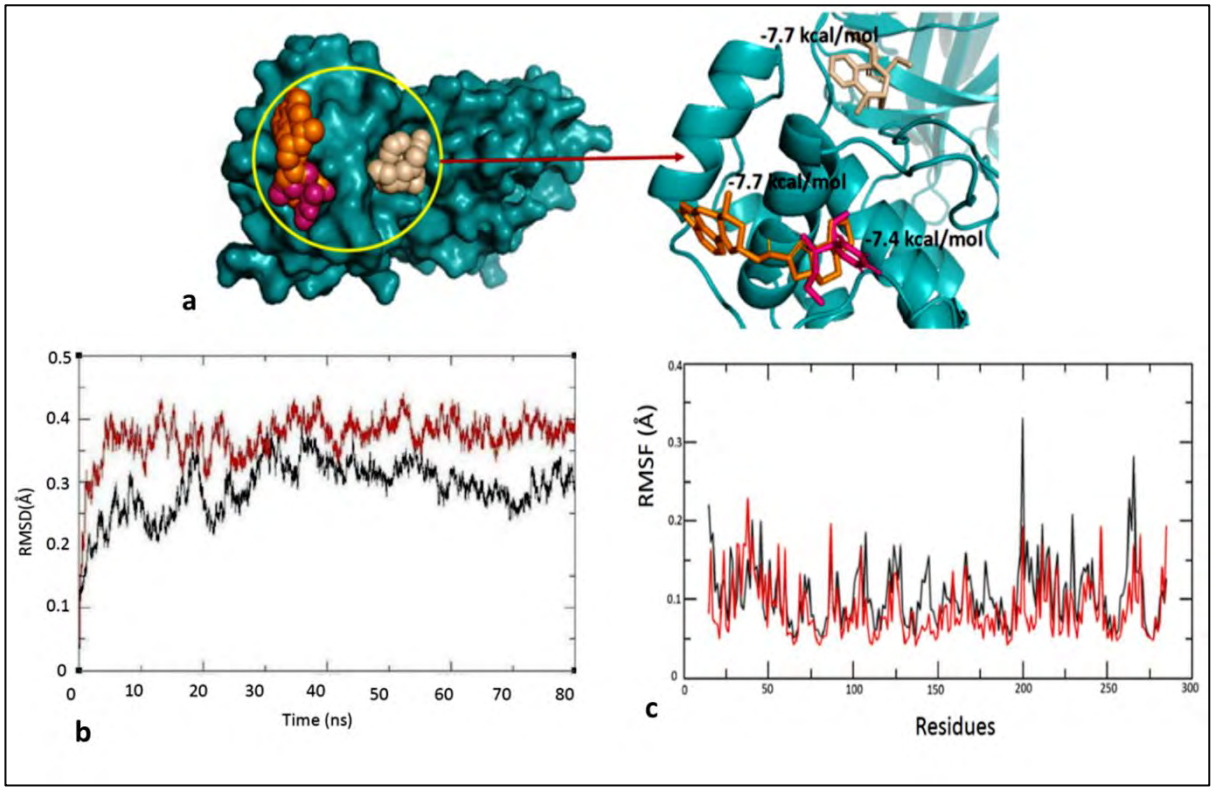

Figure1. (a). Molecular Docking analysis of Ergotaman, Vinylbital and Bis (2-ethylhexyl) phthalate along with SARS-CoV-2 M^{pro} protein. (b). RMSD value plot against time (ns) of Ergotaman-SARS-CoV-2 spike protein complex. (c) RMSF value plot against residues of Ergotaman- SARS-CoV-2 M^{pro} protein complex. (Black-Normal protein, Red-Protein Ligand Complex).

4. Conclusion

The coronavirus disease (COVID-19), first reported in Wuhan, China, in late 2019, rapidly evolved into one of the most devastating pandemics in human history, leading the World Health Organization (WHO) to declare it a global pandemic in March 2020. The unprecedented outbreak not only caused substantial morbidity and mortality worldwide but also disrupted socioeconomic structures, healthcare systems, and human mobility on an unparalleled scale. Despite remarkable global efforts toward vaccine development and antiviral drug discovery, the emergence of new SARS-CoV-2 variants and the limited efficacy of existing therapeutics against all strains continue to pose significant challenges to disease management and long-term control. Consequently, there remains an urgent need for alternative and complementary therapeutic strategies, particularly those derived from natural sources with broad antiviral potential and favorable pharmacological profiles.

In the present study, we investigated the anti-COVID-19 potential of secondary metabolites derived from a *Streptomyces* strain (Streptomyces sp. SDr-06) isolated from the tea rhizosphere soil of Darjeeling Hills, India—a unique ecological niche known for its rich microbial diversity and bioactive metabolite production. Through a comprehensive in silico analysis integrating molecular docking, dynamics simulation, and MM-GBSA energy calculations, three key metabolites—Vinylbital, Ergotaman, and Bis(2-ethylhexyl) phthalate—were identified as promising inhibitors of the SARS-CoV-

2 main protease (Mpro or 3CLpro), a critical enzyme involved in viral replication and maturation. These compounds exhibited strong binding affinities and stable protein–ligand interactions during molecular dynamics simulations, suggesting their potential to disrupt the functional conformation of Mpro.

Moreover, synergistic interaction analyses indicated that the combined inhibitory effect of these three metabolites could enhance the overall antiviral efficacy by stabilizing multiple active sites of the Mpro enzyme. Such synergism, observed in numerous natural product-based antiviral agents, reinforces the therapeutic relevance of multi-compound formulations derived from *Streptomyces* species. Notably, *Streptomyces* remains a prolific genus in the discovery of pharmacologically active metabolites, accounting for over two-thirds of clinically relevant antibiotics and several antiviral and immunomodulatory agents.

The findings from this study provide a foundation for further exploration of *Streptomyces*-derived bioactive compounds as antiviral candidates against SARS-CoV-2. However, it is essential to acknowledge that the current conclusions are based primarily on in silico approaches. Therefore, extensive in vitro and in vivo validation is required to confirm the inhibitory efficacy, cytotoxicity, pharmacokinetics, and safety of these compounds. Structural optimization, synthesis of analogs, and assessment of their antiviral activity in human cell lines and suitable animal models will be crucial next steps to establish their translational potential.

In conclusion, the study highlights the untapped potential of soil actinobacteria, particularly *Streptomyces* from the Darjeeling Hills, as a valuable source of novel antiviral compounds. The identification of Vinylbital, Ergotaman, and Bis(2-ethylhexyl) phthalate as potential Mpro inhibitors offers new insights into natural product-based antiviral drug discovery. This work underscores the significance of integrating computational, microbiological, and biochemical approaches for the rapid identification of bioactive molecules to combat emerging viral threats such as COVID-19 and future pandemics.

Authors' contributions

AS conceived and designed the study. SC, SG, and GS performed all wet lab experiments. IS carried out the bioinformatics analyses. All authors contributed to the writing and editing of the manuscript and approved the final version.

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References:

Abel R, Paredes Ramos M, Chen Q, Pérez-Sánchez H, Coluzzi F, Rocco M, Marchetti P, Mura C, Simmaco M, Bourne PE and Preissner R (2020) Computational prediction of potential inhibitors of the main protease of SARS-CoV-2. *Frontiers in Chemistry* 8:1162.

Adeyemo OM, Onilude AA and Babatola LJ (2020) Effect of production parameters and inhibitory activity of antimicrobial compounds produced by co-cultured strains of *Streptomyces xinghaiensis*-OY62 and *S. rimosus*-OG95. *Journal of King Saud University–Science* 32(1):294–301. https://doi.org/10.1016/j.jksus.2018.04.026

Aftab SO, Ghouri MZ, Masood MU, Haider Z, Khan Z, Ahmad A and Munawar N (2020) Analysis of SARS-CoV-2 RNA-dependent RNA polymerase as a potential therapeutic drug target using a computational approach. *Journal of Translational Medicine* 18(1):1–5. https://doi.org/10.1186/s12967-020-02439-0

Ahsan T, Chen J, Zhao X, Irfan M and Wu Y (2017) Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by *Streptomyces* strain KX852460 for the biological control of *Rhizoctonia solani* AG-3. *AMB Express* 7(1):1–9. https://doi.org/10.1186/s13568-017-0351-z

Alamri MA, ul Qamar MT, Mirza MU, Alqahtani SM, Froeyen M and Chen LL (2020) Discovery of human coronaviruses pan-papain-like protease inhibitors using computational approaches. *Journal of Pharmaceutical Analysis* 10(6):546–559. https://doi.org/10.1016/j.jpha.2020.08.012

Al-Khafaji K, Al-Duhaidahawi D and Taskin Tok T (2021) Using integrated computational approaches to identify safe and rapid treatment for SARS-CoV-2. *Journal of Biomolecular Structure and Dynamics* 39(9):3387–3395. https://doi.org/10.1080/07391102.2020.1764392

Amin SA, Banerjee S, Singh S, Qureshi IA, Gayen S and Jha T (2021) Structure–activity

relationship analysis of SARS-CoV-2 main protease inhibitors. *Molecular Diversity* 25:1–2.

Balachandran C, Duraipandiyan V and Ignacimuthu S (2012) Cytotoxic and antimicrobial effects of *Methylobacterium* sp. isolate ERI-135. *Asian Pacific Journal of Tropical Biomedicine* 2(9):712–716. https://doi.org/10.1016/S2221-1691(12)60215-9

Baskaran R, Mohan PM, Madanan MG, Kumar A and Palaniswami M (2015) Characterization and antimicrobial activity of *Streptomyces* sp. DOSMB-A107 from mangrove sediments. *Indian Journal of Marine Sciences* 44(5):714–723.

Chen Y, Liu Q and Guo D (2020) Emerging coronaviruses: genome structure, replication, and pathogenesis. *Journal of Medical Virology* 92(4):418–423. https://doi.org/10.1002/jmv.25681

Corre J, Lucchini JJ, Mercier GM and Crémieux A (1990) Antibacterial activity of phenethyl alcohol and membrane alterations. *Research in Microbiology* 141(4):483–497. https://doi.org/10.1016/0923-2508(90)90074-Z

Dai W, Zhang B, Jiang XM, Su H, Li J, Zhao Y, Xie X, Jin Z, Peng J, Liu F and Li C (2020) Structure-based design of antiviral drug candidates targeting SARS-CoV-2 main protease. *bioRxiv*. https://doi.org/10.1101/2020.03.25.996348

Coccolini F, Catena F, Moore EE, Ivatury R, Biffl W, Peitzman A, Coimbra R, Rizoli S, Kluger Y, Abu-Zidan FM and Ceresoli M (2016) WSES classification and guidelines for liver trauma. *World Journal of Emergency Surgery* 11(1):1–8. https://doi.org/10.1186/s12866-018-1215-7

Dholakiya RN, Kumar R, Mishra A, Mody KH and Jha B (2017) Antibacterial and antioxidant activities of novel actinobacteria. *Frontiers in Microbiology* 8:2420. https://doi.org/10.3389/fmicb.2017.02420

El-Naggar NE, El-Bindary AA, Abdel-Mogib M and Nour NS (2017) Antibiotic production by *Streptomyces anulatus* NEAE-94. *Biotechnology & Biotechnological Equipment* 31(2):418–430. https://doi.org/10.1080/13102818.2016.1276412

Gordon CJ, Tchesnokov EP, Feng JY, Porter DP and Götte M (2020) Remdesivir inhibits RNA-dependent RNA polymerase. *Journal of Biological Chemistry* 295(15):4773–4779. https://doi.org/10.1074/jbc.AC120.013056

Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S and Madden TL (2008) NCBI BLAST web interface. *Nucleic Acids Research* 36(Suppl 2):W5–W9. https://doi.org/10.1093/nar/gkn201

Li F (2016) Structure, function, and evolution of coronavirus spike proteins. *Annual Review of Virology* 3:237–261. https://doi.org/10.1146/annurev-virology-110615-042301

Pronk S, Páll S, Schulz R, Larsson P, Bjelkmar P, Apostolov R, Shirts MR, Smith JC, Kasson PM, Van der Spoel D and Hess B (2013) GROMACS molecular simulation toolkit. *Bioinformatics* 29(7):845–854. https://doi.org/10.1093/bioinformatics/btt055

Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A and Li F (2020) Cell entry mechanisms of SARS-CoV-2. *Proceedings of the National Academy of Sciences USA* 117(21):11727–11734. https://doi.org/10.1073/pnas.2003138117

Wu Z and McGoogan JM (2020) Characteristics and lessons from the COVID-19 outbreak in China. *JAMA* 323(13):1239–1242. https://doi.org/10.1001/jama.2020.2648

Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL and Chen HD (2020) Discovery of a novel coronavirus associated with pneumonia outbreak. *bioRxiv*. https://doi.org/10.1101/2020.01.22.914952