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ORIGINAL ARTICLE

In vitro and in silico anticancer potential analysis of *Streptomyces* sp. extract against human lung cancer cell line, A549

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Abstract

During our previous investigation, bioactive compounds present in the extract of *Streptomyces* sp. strain 196 were characterized using LC–MS/MS and ^{1}H NMR studies. These compounds were K-252-C aglycone indolocarbazole alkaloid, decoyinine, and cycloheximide; the study of these natural drugs against lung carcinoma is still limited. Focus of the current investigation was to study the anticancer effect of strain 196 extract on lung cancer cells (A549). During in vitro studies, anti-proliferative effect of extract was studied using MTT assay in A549 cells. Effect of extract on cell survival was further evaluated using colony assay. Cell death was qualitatively assessed using apoptosis assay. The aftereffect of extract treatment on metastatic potential of cancerous cells was studied using wound closure assay. Effect of extract on the morphology and cytoskeletal arrangement of A549 cells was studied using phalloidin staining. The extract demonstrated concentration and time-dependent cytotoxicity with IC_{50} value at 0.5 mg/ml (6 h) and 0.15 mg/ml (24 h). The proliferation and metastatic potential of cells, as characterized by MTT and migration assay, decreased over time in a concentration-dependent manner. Discrete changes in cellular morphology were noted as a result of the induced cytotoxicity. Apoptosis assay demonstrated 98.7% cell death at highest concentration of extract (1 mg/ml). During in silico studies, molecular docking revealed that strain 196 compounds are efficiently binding to mutant EGFR form (T790M/L858R) with release of binding energy (ΔG) between − 5 and − 6.9 kcal/Mol. In conclusion, strain 196 extract could be a source of therapeutic drugs to treat lung carcinoma.

Keywords *Streptomyces* · Extract · Anticancer potential · Lung cancer · A549 and WI-38 cells · In silico study

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Introduction

Lung cancer is the principal cause of mortality among malignant diseases (Rath et al. 2018). The most common subtype is non-small cell lung cancer (NSCLC) which is found in ~ 85% cases (Herbst et al. 2018), whereas small cell lung cancer (SCLC) accounts for rest of the cases (Kalemkerian et al. 2013). For their treatment radiotherapy and chemotherapy are the most widely used approaches. However, radiotherapy and chemotherapy cause adverse effects such as gastrointestinal toxicity, nephrotoxicity, oral mucositis, hepatotoxicity, cardiotoxicity, hematopoietic system injury and neurotoxicity. These noxious effects lead to a reduction in quality of life in cancer patients and ultimately results in discontinuation of therapy (Shapiro 2016; Turcotte et al. 2017; Zhang et al. 2018). Therefore, there is a need for improvement in available therapeutic options to avoid their detrimental effects.

Natural compounds are rapidly obtaining attention as a potential source of new anticancer drugs (Chin et al. 2006; Robles-Fernanzdez et al. 2013; Newman and Cragg 2014). Microorganisms are rich sources of various bioactive compounds, out of which some are authenticated to be used as therapeutic agents (Berdy 2005; Demain and Sanchez 2009). Among microbes, *Streptomyces* genus of actinobacteria has attracted scientific world due to its unmatched ability for production of bioactive compounds. Initially, Waksman and Henrici proposed the genus *Streptomyces* in 1943; at this time discovery of actinomycin from *Actinomyces antibioticus* (currently *Streptomyces antibioticus*) took place. After the identification of actinomycin D as an anticancer agent, myriad anticancer drugs such as bleomycin, anthracyclines, mitosanes, etc., have been extracted from *Streptomyces* sp. (Berdy 2005; Demain and Sanchez 2009). In recent years, the focus of research is shifting towards investigating the biosynthetic potential of *Streptomyces* sp. from underexplored habitats. Soil-derived microbes could be a potential source of compounds having pharmaceutical value.

In the present study, *Streptomyces* sp. strain 196 collected from agricultural soil of Yamuna River New Delhi, India, was extensively studied for its anticancer applications against human lung cancer cell line A549 by performing various in vitro assays and in silico studies. K-252-C aglycone indolocarbazole alkaloid, decoyinine and cycloheximide bioactive compounds were extracted in the form of crude extract using cold extraction method. Anticancer potential of strain 196 extract against human lung cancer cell line A549 was experimentally proven by conducting detailed in vitro study including cell proliferation assay, anchorage-dependent colony assay, apoptosis assay, migration-cell wound closure assay, and morphological changes: immunofluorescence. EGFR (epidermal growth factor receptor) is a transmembrane protein found on the surface of cells to which epidermal growth factors bind. This receptor plays role in the proliferation and development of cells, the aberrant expression of this receptor leads to the conversion of normal cells into the cancerous cells (Lynch et al. 2004; Hynes et al. 2005; Lu et al. 2018). Under in silico studies, molecular docking of strain 196 compounds and positive control osimertinib (Tagrisso) was performed against mutant EGFR form (PDB ID: 3W2O) having both drug resistant mutation (T790M) as well as lung cancer activating mutation (L858R). The tool used for this study was AutoDock 4.2. Furthermore, validation of docking results was performed by calculating root-mean-square deviation (RMSD) using PyMOL. In a nutshell, the results of in vitro studies revealed that extract from strain 196 possesses potential to act against human lung cancer. During in silico studies, when we compared the binding pockets of compounds from strain 196 with ligand (TAK-285) co-crystallized with mutant EGFR and positive control ligand osimertinib; it was found that binding pockets of all three compounds from strain 196 are similar to ligand co-crystallized with mutant EGFR and osimertinib. The findings of the current investigation are anticipated to offer a strong base for further development of new drugs to use in therapy having no or very fewer side effects.

Materials and methods

Actinomycete strain

Actinomycete strain under study (*Streptomyces* sp. strain 196) was isolated from agricultural soil of Yamuna River, New Delhi, India, during our earlier studies (Kapur et al. 2018a). This strain was also screened against various pathogens to assess its bioactive potential; it showed very high activity against *Bacillus cereus* MTCC 430 (Kapur et al. 2018a). 16S rRNA gene phylogenetic studies revealed that strain 196 belongs to the genus *Streptomyces* (Kapur et al. 2018b). During our recent report (Kumar et al. 2019), we have elucidated the structure of bioactive compounds present in the crude extract of strain 196.

The present investigation is focused on the anticancer potential analysis of K-252-C aglycone indolocarbazole alkaloid, decoyinine, and cycloheximide containing strain 196 extract against human lung cancer cell line A549.

Extraction of bioactive compounds

Streptomyces sp. strain 196 was streaked on yeast extract–malt extract (YM) medium plates and incubated at

28 ℃ for 15 days for production of bioactive compounds (secondary metabolites). The extraction of compounds was performed by employing the cold extraction method (Kumar et al. 2019; Schimana et al. 2000; Khanna et al. 2015). In this method, well-grown culture plates were chopped into small pieces. These pieces were transferred into flasks; ethyl acetate was added into each flask and kept for 4–5 h incubation at 200 rpm, 28 ℃ temperature in cooling incubator shaker (New Brunswick Scientific *Excella* E24, Germany). The resultant was filtered using Whatman no. 1 filter paper. Evaporation of solvent was performed by using a rotary evaporator (Buchi R-200, Switzerland). The resulted extract was weighted in two different tubes, in one tube it was dissolved in the culture medium of A549 cell line while in another tube it was dissolved in the culture medium of WI-38 cell line. In each tube, the final concentration was 2 mg/ml. Furthermore, different volumes of these main stocks were added into the media for having a series of different concentrations of extract during anticancer activity analysis against respective cell lines (A549 and WI-38).

Anticancer activity analysis

Culture of cell lines and reagents

Human lung cancer (A549) and human normal lung (WI-38) cell lines were procured from NCCS, Pune, India. A549 cells were sub-cultured in RPMI-1640 media, supplemented with 10% fetal bovine serum (v/v), and 1% antibiotic–antimycotic solution; whereas, WI-38 cells were grown on Eagle (minimum essential medium) with 2 mM L-glutamine and 1.5 g/L Na bicarbonate, 0.1 mM non-essential amino acid and 1.0 mM Na pyruvate 90%; fetal bovine serum (FBS) 10%. Cultured cells were grown at 37 °C and 5% CO_2 in a humified atmosphere.

Culture media RPMI-1640, Dulbecco's modified Eagle medium (DMEM), phosphate buffered saline (1 × PBS), 0.25% trypsin and 0.02% EDTA solution, FBS (fetal bovine serum), antibiotic–antimycotic mix solution, acridine orange (AO), ethidium bromide (EtBr), DMSO and MTT were procured from Himedia, India. Triton X-100 was purchased from Sigma-Aldrich, India. All molecular biology grade chemicals were used in the study. Alexa Fluor 594 phalloidin (A12381) was purchased from Thermo Fisher.

Cell proliferation assay

The effect of bioactive compounds containing extract of *Streptomyces* sp. strain 196 on the proliferation of human lung cancer cells (A549) and normal human lung cells (WI-38) was evaluated using MTT assay. Briefly, cells at a density of 5000 cells per well were seeded in 96 well plates and allowed to adhere overnight at 37 °C. Next day, culture media were replaced with media containing a series of different concentrations of extract ($n = 3$ per group) and incubated for another 6 and 24 h at 37 °C. Post incubation, MTT solution (20 µl of 5 mg/ml in PBS) was added, incubated for 3 h and DMSO (200 µl) was added to dissolve the formazan crystals. The absorbance (OD) was measured using a multiplate reader (Thermo Fisher, USA) at 570 nm and reference at 650 nm. Cell viability (%) was calculated using the following equation (Zhang et al. 2017; Kumar et al. 2018; Kumar et al. 2019a, b)

$$\%Cell\ viability = \frac{Absorbance_{treated} - Absorbance_{blank}}{Absorbance_{control} - Absorbance_{blank}} \times 100$$

where control is cells without extract and blank is only media.

Anchorage-dependent colony assay

A549 cancer cells were seeded in 60-mm culture dish at a density of 500 cells per dish. Subsequent to cell attachment, culture media containing different concentrations of extract (0.001 to 1 mg/ml) were added for 24 h. In the post-incubation period, extract containing media were aspirated and fresh media were added to each dish for an additional 7 days. After the respective time point, cells were washed with 1 × PBS (phosphate buffered saline), fixed in 4% paraformaldehyde for 20 min and stained using crystal violet solution (0.4% w/v). The excess solution was washed and colony formation was computed based on visual examination (Washio et al. 2018).

Apoptosis assay

In this assay, acridine orange–ethidium bromide (AO–EtBr) staining was used for qualitative assessment of apoptosis in the treated cancer cells (Kasibhatla et al. 2006; Kumar and Tiku 2018). AO stains both live as well as dead cells and gives green fluorescence, whereas EtBr stains dead cells and gives red fluorescence. Live cells display uniform green fluorescence, while apoptotic and dead cells display orange and red color, respectively. Subsequently, to 24 h of incubation with extract, cells were harvested and single-cell suspension (8 µl) was mixed with AO–EtBr stain (1 mg/ml; 1:1 v/v) and incubated for 2 min. The cells were visualized using a fluorescence microscope (Make- Nikon ECLIPSE Ti-S, Japan) at 10 × magnification.

Migration-cell wound closure assay

A549 cells at a density of 5×10^5 cells were seeded in a 6-well plate and allowed to grow till 80% confluency. Once

confluent, a scratch was made in each well with the help of 200 µl micropipette tip and media containing various concentrations of extract (0.001 to 1 mg/ml) was added. The changes in the migration-cell wound closure were noted at 0 h, 24 h and 48 h using a bright-field microscope (Make-Nikon ECLIPSE Ti-S) at 10 × magnification and analyzed using ImageJ software (Washio et al. 2018; Garcia et al. 2016).

Morphological changes: immunofluorescence

For the study the effect of the extract on the morphology and cytoskeletal arrangement of A549 cells, phalloidin staining was performed (Washio et al. 2018; Nersesian et al. 2018). Briefly, cells incubated with different concentrations of extract for 24 h were fixed in 4% paraformaldehyde, using 0.05% Triton X-100 for permeabilization, and blocked with 5% BSA. Alexa Fluor 594 Phalloidin (A12381, 1:100 dilution) was added for 40 min followed by nuclear staining with DAPI. Mounted slides were examined under a fluorescent microscope (Make- Nikon ECLIPSE Ti-S, Tokyo, Japan) at 60 × magnification.

Statistical analysis

Each experimental group consisted of 3 replicates. Statistical analysis was performed using one-way ANOVA and two-way ANOVA, followed by Tukey post hoc (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ were considered statistically significant). Data are represented as mean ± standard error of the mean.

Molecular docking

Nearly 70–80% cases of non-small cell lung cancer (NSCLC) are due to activating mutation in EGFR tyrosine kinase domain (Lynch et al. 2004). Molecular docking studies were performed to check whether three compounds from strain 196 can bind to mutant EGFR.

Molecular docking of compounds K-252-C aglycone indolocarbazole alkaloid, decoyinine, and cycloheximide present in strain 196 extract was performed against mutant (PDB ID: 3W2O) form of epidermal growth factor receptor (EGFR) tyrosine kinase domain by taking osimertinib (Tagrisso) as positive control. After docking, comparison of the binding pockets of compounds from strain 196 with ligand (TAK-285) co-crystallized with mutant EGFR and positive control ligand osimertinib was done. AutoDock 4.2 with several supporting tools such as MGL tools, Cygwin, Discovery Studio Visualizer, Binary files, and Java was used to perform the docking. The steps for the usage of AutoDock 4.2 were followed as per the protocol given by Rizvi et al. (2013). For validation of docking results root-mean-square deviation (RMSD) was calculated using PyMOL.

Results

Actinomycete strain

Streptomyces sp. strain 196 was grown on YM medium at 28 ℃, after 5 days it showed white colored aerial spores. This grown culture was used for further studies reported in this paper.

Extraction of bioactive compounds

The cold extraction method was employed for isolation of bioactive compounds using ethyl acetate as solvent. Solvent evaporation in rotary evaporator resulted in bioactive compounds containing semi-solid residue (extract). Furthermore, this extract was dissolved in the culture media of cell lines (A549 and WI-38) and used in the anticancer activity analysis.

Anticancer activity analysis

Cell proliferation assay

In vitro anti-proliferative effect of the extract on A549 human lung cancer cells demonstrated a decrease in cell viability in a dose and time-dependent manner (Fig. 1a). Reduction in the viability of cancer cells was initiated at 0.1 mg/ml after 24 h of treatment; while after 6 h, the extract was effective on cells at the only higher dose (Fig. 1a). Extract inhibited proliferation of cells up to 50% at 0.15 mg/ml (24 h) and 0.5 mg/ml (6 h). This suggests that the anti-proliferative effect of the extract was effective after 6 h of treatment. Normal human lung cells (WI-38) treated with the same doses of the extract showed no significant reduction in cell viability as compared to extract-treated cancer cells (Fig. 1b). This suggests that the anti-proliferative effect of the drug specifically restricted to cancer cells only.

Anchorage-dependent colony assay

The effect of the extract on cell survival was further evaluated using colony assay, where a group of at least 50 cells

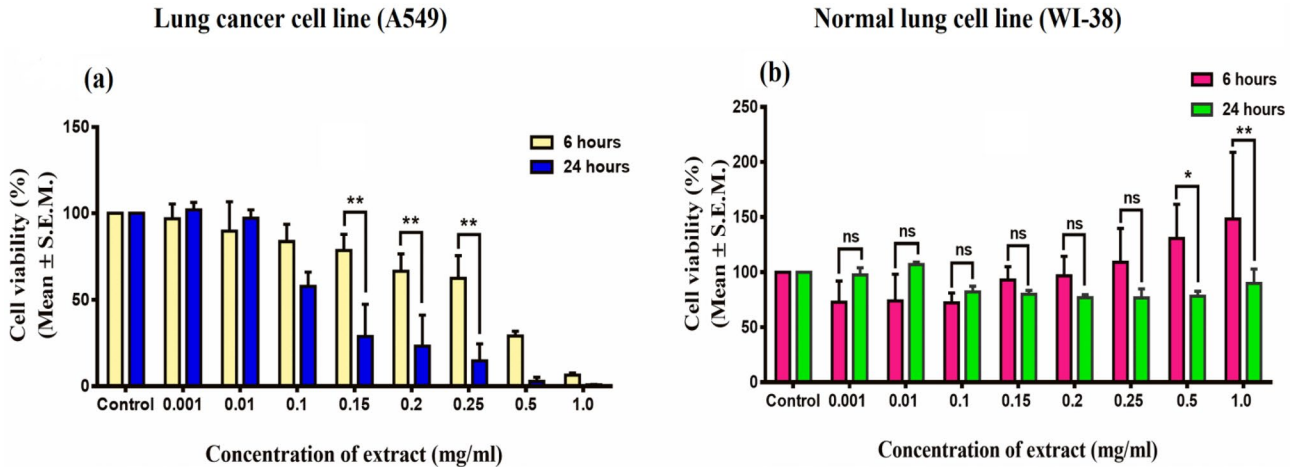

Fig. 1 **a** Effect of different concentrations of the strain 196 extract on A549 cell viability by MTT assay, data represented as mean ± standard error of mean of three independent experiments (**$p < 0.01$, the significant difference in between treatment group compared over a period of time). **b** Effect of different concentrations of the strain 196 extract on WI-38 (normal lung cell line) cell viability by MTT assay. Data represented as mean ± standard error of the mean of three independent experiments (**$p < 0.01$, the significant difference in between treatment group compared over a period of time)

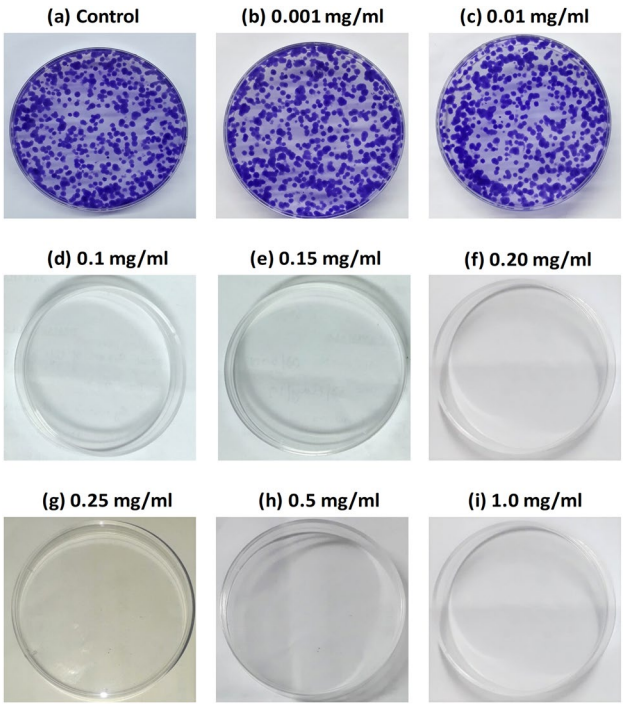

Fig. 2 Effect of different concentrations of strain 196 extract on A549 colony formation. A549 cancer cells were seeded in 60-mm culture dish at a density of 500 cells per dish. After cell attachment, culture media containing different concentrations of extract (0.001 to 1 mg/ml) were added for 24 h. After incubation period, extract containing media were aspirated and fresh media were added to each dish for an additional 7 days. After the respective time point, cells were washed with 1 × PBS, fixed in 4% paraformaldehyde for 20 min and stained using crystal violet solution (0.4% w/v). The excess solution was washed and colony formation was computed based on visual examination

was referred to as a colony. As clearly shown in Fig. 2, 0.001 mg/ml and 0.01 mg/ml extract concentrations showed similar colonies in terms of number and size as compared to the control group. Whereas, higher concentrations of extract from 0.1–1 mg/ml; significantly diminished the number of colonies in a dose-dependent manner.

Apoptosis assay

Cell death was qualitatively assessed using AO–EtBr staining on treated cells. As can be seen in Fig. 3, control (untreated cells) displayed green fluorescence suggesting that all cells were live. In contrast, cells demonstrated relatively higher death as the concentration of extract increased (Fig. 3a). At higher concentration (1 mg/ml), 98.7% cell death was observed (Fig. 3b).

Migration-cell wound closure assay

The aftereffect of extract treatment on the metastatic potential of cancerous cells was studied using wound closure assay. At 0.01–1 mg/ml, the extract demonstrated a clear dose-dependent reduction in the motility of cancer cells. The rate of wound healing of control cells (untreated) was 57% and 92% after 24 h and 48 h, respectively (Fig. 4a, b). In contrast, only 15% and 18% of cells migrated after 24 h and 48 h, respectively, at 0.1 mg/ml. A significant reduction in cell motility was observed at concentrations below the anti-proliferative inhibitory concentration (IC_{50}) values. These results suggest that the extract inhibited the metastatic potential of the cancerous cells A549 in a dose and time-dependent manner.

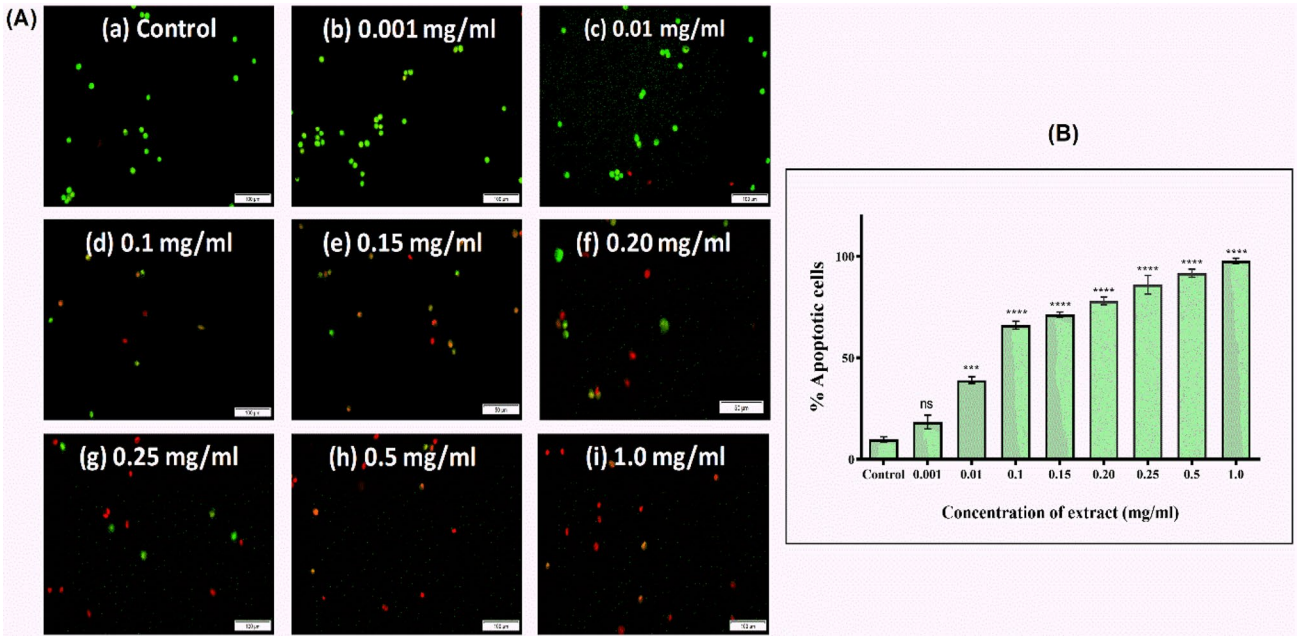

Fig. 3 Extract induced apoptosis in A549 cells as detected using AO–EtBr. **a** Effect of different concentrations of strain 196 extract on A549 cells. Merged image shows live (green), apoptotic (orange) and dead cells (red). Cells were visualized at 10X magnification; scale bar 100 µm. **b** Representative graph of percent total dead cells. Data represented as mean ± standard error of the mean. $*p < 0.05$; $**p < 0.01$, $***p < 0.001$ compared to control group. ns represents non-significant

Morphological changes: immunofluorescence

The morphology of cancer cells studied using phalloidin staining demonstrated dose-dependent changes in morphology. At lower doses (0.001 mg/ml and 0.01 mg/ml), cell morphology was similar to control group (Fig. 5), while higher doses of extract triggered phenotypical changes in terms of rounded morphology, reduction in cell size, cytoskeleton aggregation at the periphery and cytoskeleton disruption after 24 h (Fig. 5).

Molecular docking

The compounds from strain 196 were used during molecular docking against mutant EGFR (T790M/L858R). The results revealed that K-252-C aglycone indolocarbazole alkaloid release binding energy (ΔG) of − 6.9 kcal/Mol with mutant EGFR (Fig. 6a and Table 1). In case of decoyinine released ΔG with mutant EGFR was -5.17 kcal/Mol (Fig. 6b and Table 1); whereas, in cycloheximide the released ΔG with mutant EGFR was -5.63 kcal/Mol (Fig. 6c and Table 1). The positive control osimertinib released ΔG -6.28 with mutant EGFR (Fig. 7 and Table 2). The docking results suggested that the compounds present in the extract from strain 196 are efficiently binding to the mutated site of EGFR receptor (T790M/L858R). When we manually compared the binding pockets of compounds from strain 196 with ligand (TAK-285) co-crystallized with mutant EGFR and positive control ligand osimertinib; it was found that binding pockets of all three compounds from strain 196 are similar to ligand co-crystallized with mutant EGFR and osimertinib. All the ligands (i.e., experimental and controls) were observed to bind the pocket which contains mutated site 'Met790' in the receptor. Validation of docking results using PyMOL revealed that the complexes K-252-C aglycone indolocarbazole alkaloid + mutant EGFR, decoyinine + mutant EGFR, cycloheximide + mutant EGFR, and osimertinib (positive control) + mutant EGFR when superimposed on mutant

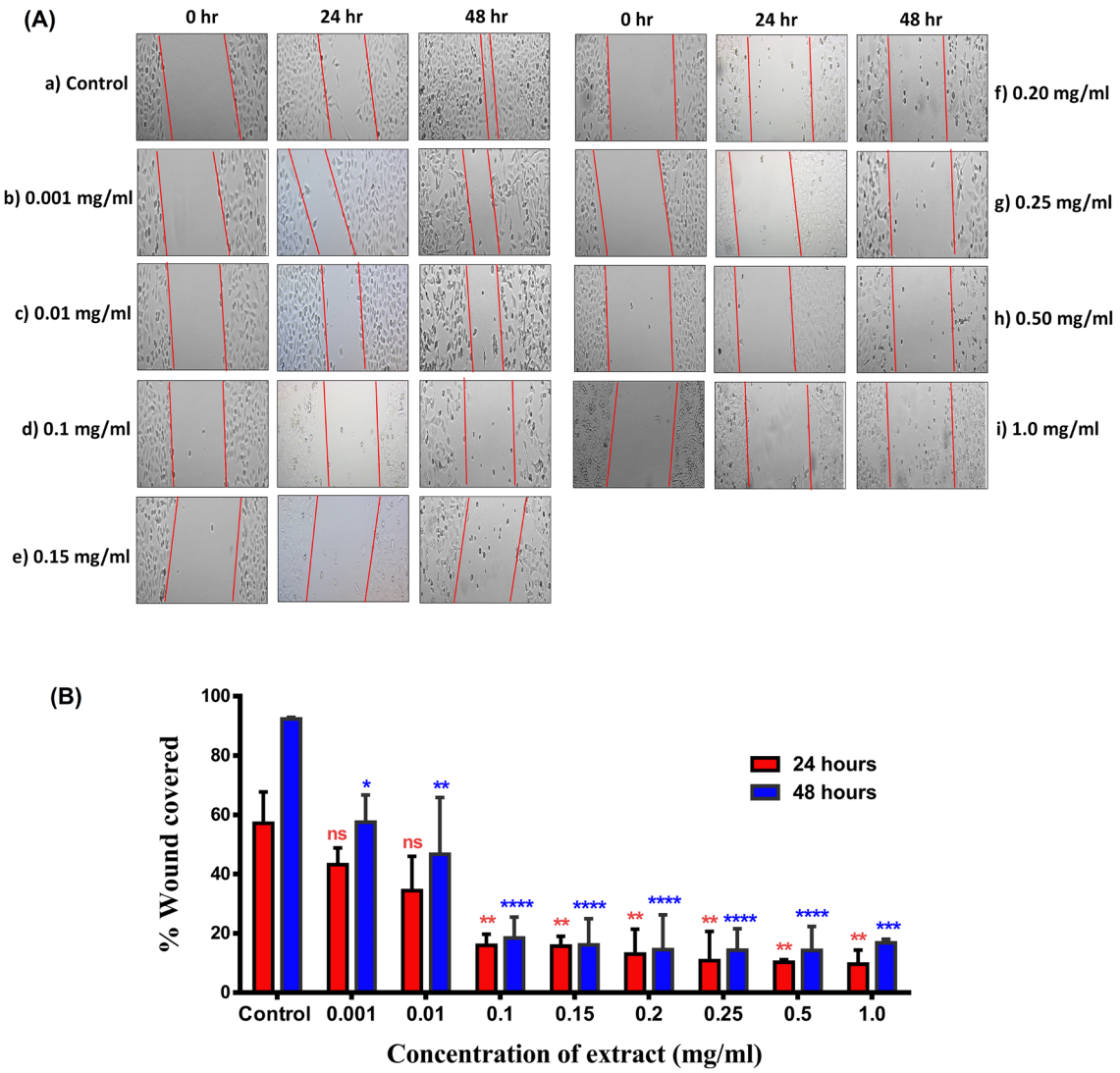

Fig. 4 Effect of extract on cell motility. **a** Images show the effect of different concentrations of strain 196 extract on A549 cells migration by monolayer migration-cell wound closure assay at different time points (0, 24 and 48 h). **b** Representative bar plot is shown. The measurements are from the wound made on A549 cultured cells in the presence of control (untreated) and extract-treated. Data represented as mean ± standard error of the mean. *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$ compared to control group

EGFR co-crystallized with ligand TAK-285, each showed RMSD value 0 (Fig. 8). Thus, validation results suggested that compounds from strain 196 are efficiently binding to the mutated site of EGFR receptor (T790M/L858R).

Discussion

In recent years, natural products from actinomycetes have received unprecedented attention because of their wide range of biological activities such as antitumor, antimicrobials, antihypertensive, immune-suppressant, etc. Actinomycetes belonging to *Streptomyces* genus were found to possess exceptional bioactive natural products synthesis

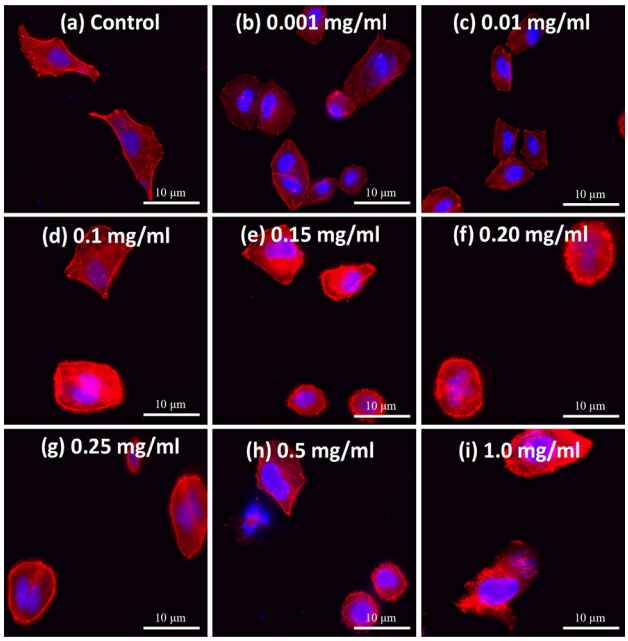

Fig. 5 Phalloidin staining showing F-actin morphology of control and strain 196 extract-treated A549 cells. **a** Control (untreated A549 cells). **b–i** A549 cells treated with different concentrations of extract. Red color shows phalloidin-stained actin filaments, blue color shows DAPI-stained nuclei. Cells were visualized at 60 × magnification

abilities. Thus, the bacteria from this genus are worth to be investigated.

In this study, K-252-C aglycone indolocarbazole alkaloid, decoyinine, and cycloheximide containing extract from *Streptomyces* sp. strain 196 was studied for its anticancer potential against lung cancer cell line A549. Cytotoxicity studies have played a pivotal role in the discovery of new anticancer drugs over the past three decades. In the present study, the cytotoxic activity of strain 196 extract revealed that strain 196 extract suppressed proliferation of cancer cells in a concentration and time-dependent way. It was observed that after 24 h of the incubation period, strain 196 extract-treated cells at a concentration of 0.15 mg/ml showed only 28% cell viability. Moreover, the cytotoxic study evaluated on normal human lung cells (WI-38) revealed no significant reduction in cell viability compared to cancer cells (A549). Therefore, strain 196 extract could potentially be used as anticancer therapeutics for human.

Cytotoxic mechanism of natural bioactive compounds, interfere with basic cellular functions such as cell cycle, apoptosis, inflammation, angiogenesis, invasion and metastasis. Many anticancer drugs from marine organisms cause cytotoxicity and cell death through the induction of apoptosis. In the present study, the long-term cytotoxic effect of the extract on the proliferation of a single cell into a viable colony was investigated and results suggested that extract significantly reduced clonogenic capability of A549 cells in a concentration-dependent manner. Strain 196 extract-treated cells induced apoptosis further confirmed the concentration-dependent cytotoxic effect of the extract on A549 cells. As the concentration of extract increased, cell death also increased.

Besides cell death, the metastatic potential of cells was decreased in a concentration-dependent manner. It is noteworthy that beyond a particular concentration (0.1 mg/ml), the extract showed a similar cytotoxic effect on cells. A significant reduction in cancer cell motility was observed at a concentration below the anti-proliferative inhibitory concentration (IC_{50}) values. This cytotoxic behavior of strain 196 extract was reflected in the phenotypical changes of cells in terms of nuclear morphology, reduction in cell size, cytoskeleton aggregation, loss of normal nuclear architecture and cytoskeleton disruption indicating deterioration of cells, which was not observed in untreated cells.

Moreover, overexpression of EGFR is directly related to lung cancer development, the mutation (L858R) in EGFR tyrosine kinase domain has been detected as the driver for the development of non-small cell lung cancer (NSCLC) (Hynes et al. 2005). In recent years, therapy with EGFR tyrosine kinase inhibitors (EGFR-TKI) has played a vital role in the improvement of survival of NSCLC patients with mutations in EGFR (Lu et al. 2018). Gefitnib and erlotinib are the first generation inhibitors applied clinically and provided a great response in the treatment of NSCLC patients with mutations in EGFR. After 9–14 months, in these types of cases, an acquired resistance observed against gefitinib and erlotinib (Lu et al. 2018). The secondary mutation T790M (threonine^{790} to methionine^{790}) in EGFR is responsible for 55–70% acquired resistance in NSCLC patients with EGFR mutations. Some second-generation inhibitors as afatinib and dacomitinib have been developed to treat resistance caused by EGFR (T790M) mutation (Lu et al. 2018).

In the present investigation, molecular docking studies were performed to check whether compounds from strain 196 can bind to mutant EGFR. Results suggested that the compounds K-252-C aglycone indolocarbazole alkaloid, decoyinine and cycloheximide can efficiently bind to the

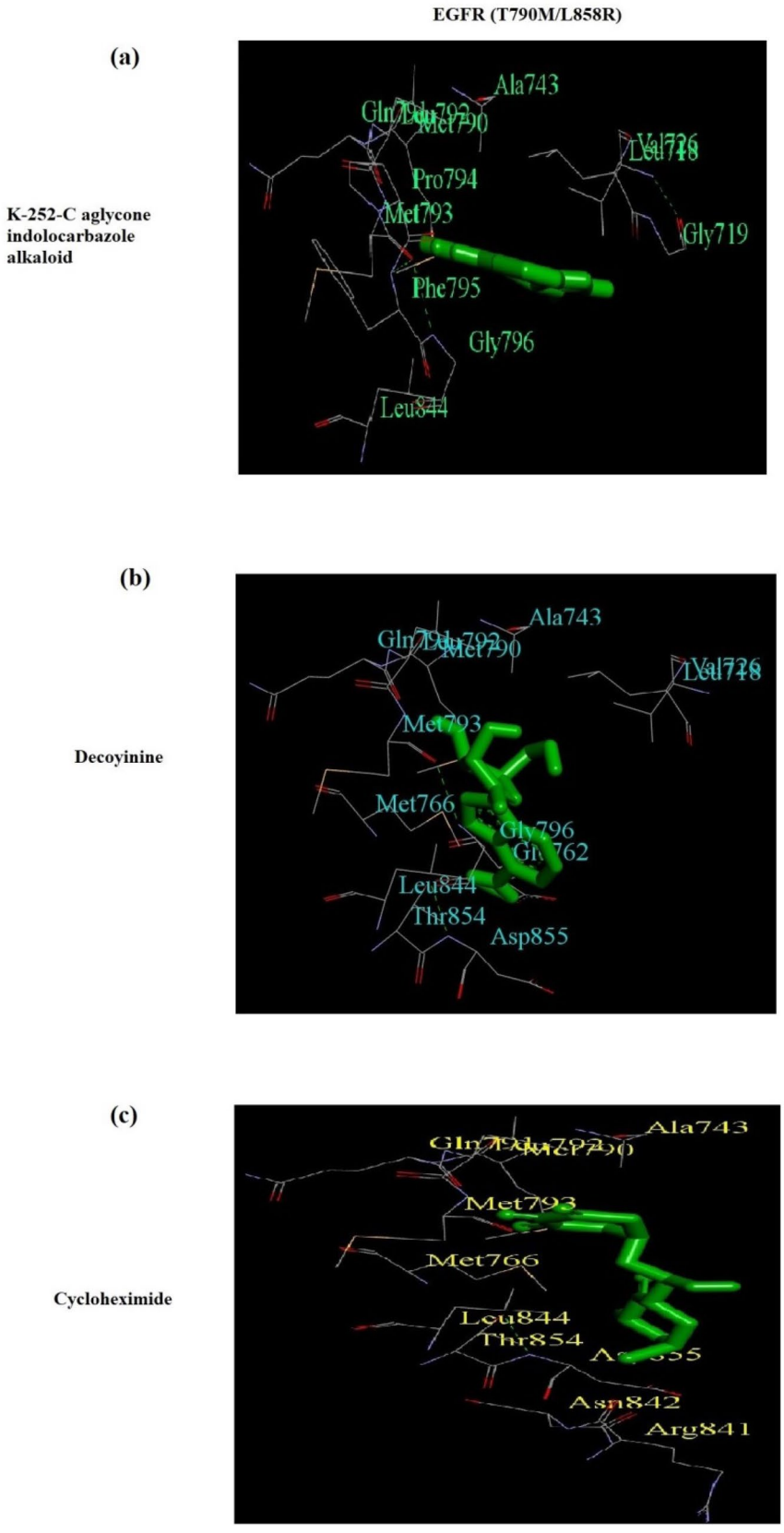

Fig. 6 Molecular docking analysis of **a** K-252-C aglycone indolocarbazole, **b** decoyinine and **c** cycloheximide against mutant form of EGFR (T790M/L858R) tyrosine kinase domain

Table 1 Molecular docking analysis of K-252-C aglycone indolocarbazole alkaloid, decoyinine, and cycloheximide compounds against mutant EGFR (T790M/L858R)

Bioactive compound	Receptor	Binding energy (ΔG) kcal/mol	Interacting amino acids
K-252-C-aglycone indolocarbazole alkaloid	EGFR (T790M/L858R mutant)	− 6.9	Gln791, Leu792, Met790, Ala743, Pro794, Met793, Val726, Leu718, Phe795, Gly719, Gly796, Leu844
Decoyinine	EGFR (T790M/L858R mutant)	− 5.17	Gln791, Leu792, Met790, Ala743, Met793, Val726, Leu718, Met766, Gly796, Gln762, Leu844, Thr854, Asp855
Cycloheximide	EGFR (T790M/L858R mutant)	− 5.63	Gln791, Leu792, Met790, Ala743, Met793, Met766, Leu844, Thr854, Asp655, Asn842, Arg841

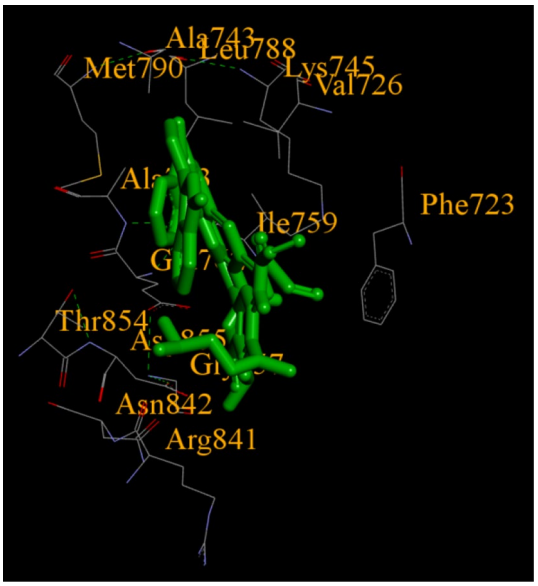

Fig. 7 Molecular docking analysis of osimertinib (positive control) against mutant form of EGFR (T790M/L858R)

EGFR having T790M/L858R mutations. When we compared the binding pockets of compounds from strain 196 with ligand co-crystallized with mutant EGFR and positive control ligand osimertinib; it was found that binding pockets of all three compounds from strain 196 are similar to ligand co-crystallized with mutant EGFR and osimertinib. All the ligands (i.e., experimental and controls) were observed to bind the pocket which contains mutated site 'Met790' in the receptor. Validation of molecular docking data suggested that the binding of these compounds on mutated EGFR is efficient as the RMSD value is 0 in each case. So, it can be suggested that there was no forced docking. Docking results were compared with positive control osimertinib which is a third-generation inhibitor of mutant EGFR. It was suggested that K-252-C aglycone indolocarbazole alkaloid binding mutant EGFR more efficiently than the osimertinib; whereas, decoyinine and cycloheximide were found to have less efficient binding in contrast to osimertinib.

Altogether, these findings suggested that strain 196 extract may serve as a potential therapeutic agent against lung cancer. Overall our study warrants further investigation of the extract for subsequent *in vivo* and clinical trials studies to establish the strain 196 extract as a potentially safe agent for the treatment of lung cancer.

Conclusion

K-252-C aglycone indolocarbazole alkaloid, decoyinine, and cycloheximide containing strain 196 extract reduced A549 cell viability in a dose and time-dependent manner. The extract influenced the proliferation, colony formation, and migration of A549 cells in the respective assays. So, the results of in vitro anticancer effect analysis of strain 196 extract revealed that it has high cytotoxicity against human lung cancer cells (A549); whereas normal human lung cells (WI-38) treated with the same doses of the extract showed no significant reduction in cell viability. In silico studies (molecular docking) suggested that compounds present in extract from strain 196 bind efficiently to mutant EGFR (T790M/L858R).

Table 2 Molecular docking analysis of osimertinib (positive control) against mutant EGFR (T790M/L858R)

Tyrosine kinase inhibitor	Receptor	Binding energy (ΔG) kcal/mol	Interacting amino acids
Osimertinib (Tagrisso)	EGFR (T790M/L858R mutant)	− 6.28	Leu788, Met790, Lys745, Ala743, Val726, Ala763, Ile759, Phe723, Glu762, Thr854, Asp855, Gly857, Asn842, Arg841

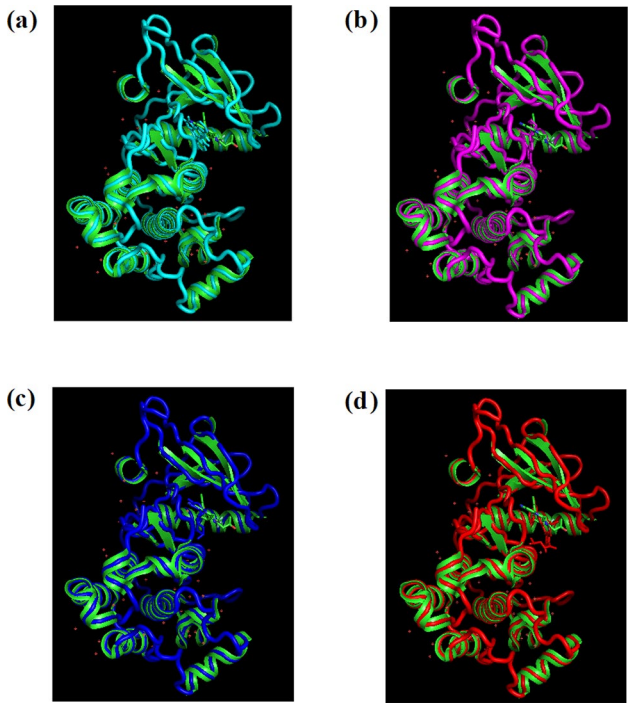

Fig. 8 Calculation of root-mean-square deviation (RMSD) by superimposing receptor–ligand complex on receptor co-crystallized with ligand TAK-285 using PyMOL: **a** K-252-C aglycone indolocarbazole alkaloid + EGFR (T790M/L858R) complex superimposed on EGFR (T790M/L858R) co-crystallized with ligand TAK-285. **b** Decoyinine + EGFR (T790M/L858R) complex superimposed on EGFR (T790M/L858R) co-crystallized with ligand TAK-285. **c** Cycloheximide + EGFR (T790M/L858R) complex superimposed on EGFR (T790M/L858R) co-crystallized with ligand TAK-285. **d** Osimertinib (positive control) + EGFR (T790M/L858R) complex superimposed on EGFR (T790M/L858R) co-crystallized with ligand TAK-285

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Author contributions PK, AC, MK, BKK and MKK conceived and designed the experiments. PK and AC performed all experiments. PK analyzed the data reported in this study. PK, AC, MK, BKK, AK, RS, and MKK contributed in writing. All authors read and approved the manuscript.

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Declarations

Conflict of interests The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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