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Research Article
Acute Anti-Cancer Activity of Crude Extracts from two Endophytic Fungi *Chaetomium cochliodes* and *Penicillium* Sp. in Cancer Cell Lines and Mice

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Abstract

Background and Objective: The discovery of novel natural lead compounds is the key issue in searching for new anti-tumour drugs. Endophytic fungi have been proved to be a promising source for new bioactive compounds with potential application in medicine. This study aimed to investigate the activity of antiproliferation, cell cycle arrest, induction of apoptosis and anti-tumour growth of the crude extracts from the two endophytic fungi, *Chaetomium cochliodes* and *Penicillium* sp. **Materials and Methods:** The crude extracts of the fungi *Chaetomium cochliodes* and *Penicillium* sp., were obtained by enrichment and extraction *in vitro*. The proliferation, cell cycle and apoptosis were evaluated the killing effects of tumour cells and cytotoxicity in Huh7 and MGC-803 cell lines. The effect of the extract *in vivo* was studied in a subcutaneously implanted tumour model in C57BL/6 mice. **Results:** The cancer cell survival rate was almost less than 10% after the treatment of the crude extracts of *Chaetomium cochliodes* and *Penicillium* sp., by using a cell proliferation assay. Through the apoptosis assay, the crude extracts from *Chaetomium cochliodes* and *Penicillium* sp., significantly increased the number of apoptosis compared with the control group. The crude extract from *Chaetomium cochliodes* mainly induced G2/M phase arrest of MGC-803 cells, which might be contributing to the apoptosis. Importantly, these two crude extracts significantly reduced the volume and size of subcutaneous transplanted tumours after treatment in transplanted tumour models. **Conclusion:** The present study demonstrated that the extracts obtained from *Chaetomium cochliodes* and *Penicillium* sp., are promising materials for tumour treatment. These results expand the medicinal value of *Panax notoginseng* and *Taxus media* and provide a new direction for its clinical application.

Key words: Crude extracts, anti-cancer activity, endophytic fungi, *Chaetomium cochliodes*, *Penicillium* sp.

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Natural products, with novel structures and unique activity, played a very important role in cancer treatment. During the period 1980 to 2019, there were 185 anti-tumour small molecule drugs approved worldwide, of which 120 were related to natural products[1]. However, in recent years, one of the biggest bottlenecks restricting the development of anti-tumour drugs is the lack of quality source molecules. Therefore, the discovery of novel natural lead compounds is the key issue in searching for new anti-tumour drugs.

Plants, including traditional Asian herbs, are a major source for the discovery of anti-tumour small molecule drugs. Plant-derived small molecule drugs such as paclitaxel, honokiol, camptothecin, vinblastine and podophyllotoxin are well-known anti-tumour drugs, some of which have been used clinically and have achieved good results[2]. Among them, paclitaxel has been proved curative effects on many tumours. More importantly, it is the first-line drug for the treatment of ovarian cancer and breast cancer[2]. However, due to the low content of these active small molecules in plants, a large number of plants was consumed to generate sufficient drugs. For example, producing 1 kg of paclitaxel needs 3,000-4,000 60-year-old trees of *Taxus*. Since the advent of paclitaxel, the world's natural yew has been devastated and over-exploited and the destruction of native resources is making the yew plant resources endangered. Therefore, the development of new sources of anti-cancer small molecule drugs is crucial.

Endophytic fungi with distinct physiological and biochemical characteristics have been proved to be a promising source for new bioactive compounds with potential application in medicine. According to a previous study, the discovery rate of new compounds in the endophytic fungi (~51%) is much higher than that of soil microorganisms (~38%)[3]. Many of these compounds from the endophytic fungi have anti-tumor activity, such as cell relaxin chaetoglobosin U from *Chaetomium globosum* IFB-E019 associated with *Imperata cylindrica* displaying significant inhibitory activity against human nasopharyngeal carcinoma KB cells[4], a new alkaloid, 9-deacetoxyfumigaclavine C from *Aspergillus fumigatus* of associated with *Cynodon dactylon* exhibiting significant inhibitory activity against human leukemia cells K562[5], a new alkaloid, IFB-E015 from *Chaetomium* sp., associated with *Adenophora axilliflora* possessing significant inhibitory activity on human leukemia cell line K562 and colon cancer SW1116 cells[6], a new diterpenoid type of periconicin from *Periconia atropurpurea* associated with *Xylopia aromatic* showing significant inhibitory activity against mammalian cell line cervical cancer (HeLa) and Chinese hamster ovary (CHO)[7], a new macrolide compound brefeldin A from *Acremonium* sp., associated with *Knema laurina* having significant inhibitory activity on human tumor cells KB, BC-1 and NCI- H187[8], two new polyketones compound is associated with *Aegiceras corniculatum* exhibiting significant inhibitory activity against human lung cancer cells and leukemia cells respectively[9]. Therefore, screening new anti-tumour small-molecule drugs from endophytic fungi is worth a try.

Panax notoginseng has been widely used in traditional Chinese medicine to treat stroke, ischemic heart and brain diseases. The treatment of *P. notoginseng* could prevent and treat ischemic cerebrovascular diseases[10], promote neuronal plasticity, inhibit neuronal apoptosis[11] and so on. According to recent studies, both *Panax notoginseng* and *Taxus media* have antitumor activities[12,13]. *Taxus* species are also traditional medicinal plants, the shoots and leaves have been used to treat kidney disease and rheumatism[14]. Multiple studies showed that *Taxus* species have anticancer activities, such as treating pancreatic cancer with water decoctions of *Taxus* cuspidate[15] and treating non-small cell lung cancer with aqueous extracts[16]. Therefore, it's important to explore the new anti-tumour endophytic fungi from these two medicinal plants.

In the present study, two endophytic fungi were isolated and cultured from these herbs. The anti-cancer activity of the crude extracts from two fungi was further tested by cells proliferation assay, cell cycle assay and apoptosis assay.

MATERIALS AND METHODS

Study area: This research was carried out at the Institute of Pathology and Southwest Cancer Center, Southwest Hospital, Army Medical University, Biobank and Clinical Research Center, Chongqing Public Health Medical Center, Chongqing, China in September, 2018. The crude extracts were prepared at the School of Pharmaceutical Sciences, South-Central University for Nationalities, Wuhan, China.

Fungal material: The fungal strains, XL-1325 and MPT-426, were isolated from the stems of *Panax notoginseng* and the roots of *Taxus media*, respectively. Two isolates were identified to be *Chaetomium cochliodes* (XL-1325) and *Penicillium* sp., (MPT-426) by an analysis of the ITS region of the rDNA. The voucher specimens have been deposited at the School of Pharmaceutical Sciences, South-Central University for Nationalities. Above two fungi were inoculated into the flasks with 200 mL malt extract broth (MEB) medium (sucrose 2%, malt extract 2%, peptone 0.1%) on a rotary shaker at 30°C,

180 rpm for 30 days, respectively. The harvested fermentation material was extracted three times with ethyl acetate at room temperature. Then, the crude extracts of *Chaetomium cochliodes* (XL-1325) and *Penicillium* sp. (MPT-426) were prepared from the organic solvent by a lyophilization method. Each crude extract was dissolved into the solvent of DMSO for the anticancer activity test.

Animal maintenance and treatments: The mice were purchased and cared for from the Center of Animals (Third Military Medical University, Chongqing, China). Mice studies were performed by the guidelines of the Research Council and Animal Use and Care Committee of Army Medical University. 5 weeks female C57BL/6 mice were used as the recipient mice.

Cells and culture: Human hepatocellular carcinoma cell line Huh-7, Human Glioma Cell line LN229, Human breast cancer cell line MDA231, Human gastric carcinoma cell line MGC-803 and Mouse glioma cell line GL261 were obtained from Shanghai Cell Research Institute of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (Gibco) at 37°C with 5% CO_2.

Dissolution and dilution of drugs: The crude extracts of *Chaetomium cochliodes* and *Penicillium* sp., were dissolved and diluted in DMSO (Sigma) at the concentration of 0.5 mg μL^{-1}. Store working aliquots at -20°C.

Cell viability assays: Cancer cells were harvested and counted during logarithmic-phase growth and 2000 cells/per well were seeded in a 96-well plate in 100 µL of growth media. The cells were cultured overnight for 24 hrs with the medium of serum-free DMEM at 37°C in a humidified 5% CO_2 atmosphere. Then, the crude extracts of *Chaetomium cochliodes* and *Penicillium* sp., were diluted in a complete medium to concentrations of 0, 0.5, 1, 2.5, 5, 10, 15, 20 and 25 µg mL^{-1} separately, 100 µL of these working solutions were changed in 96-well plate. The plate was then placed back in the 5% CO_2 incubator at 37°C for an additional 48 hrs. Then 10 µL Cell Counting Kit-8 (Beyotime, China) was added to each well and the plates were incubated for an additional 3 hrs at 37°C. Measure the absorbance at 450 nm by a microplate reader. The IC_{50} value (half maximal inhibitory concentration) was determined by the sigmoidal curve.

Cell proliferation assay: Cancer cells were seeded into 96-well flat-bottomed plates with the density of 2×10^3 cells/well for Huh-7 and 1×10^3 cells/well for MGC-803. The cells were suspended in the serum-free DMEM medium and cultured overnight. After 8 hrs, replace the complete medium with candidate extracts in concentrations corresponding to the IC_{50} value for each cell line or DMSO (control) at 37°C with 5% CO_2. And continuous culture was performed for 5 days. At the indicated intervals, the 450 nm absorbance was measured by a microplate reader (Thermo Scientific). The details were proceeded according to the previous article published by the group[17].

Cell cycle assay: Cancer cells Huh-7 (2×10^5 vcells/well) and MGC-803 (1×10^5 cells/well) were seeded into 6-well flat-bottomed plates. The cells were suspended with serum-free DMEM and cultured overnight at 37°C with 5% CO_2. After 8 hrs, the cells were cultured with a fresh medium containing the candidate extracts in concentrations corresponding to the IC_{50} value for each cell line. DMSO was used as the solution control. After 24 hrs, both attached and detached cells were harvested via trypsinization, washed with PBS and fixed in 70% ethanol overnight at 4°C. The pre-treatment of cells was carried out and centrifuged for 5 min to go to the supernatant at 1000 rpm, then resuspended with cold PBS and centrifuged to go to the supernatant. The cell pellet was using Cell Cycle (Beyotime, China) following the manufacturer's instructions. Cells were analyzed by ow cytometer BD FACS Aria II. Quantification of DNA content was performed using Flowing software and the cell cycle phase distribution was shown by histograms.

Apoptosis analysis: The Annexin V/FITC assay was performed using the Annexin V/FITC apoptosis detecting kit (Beyotime, China) to analyze the potential of tested extracts in causing apoptosis. The cells were seeded in 6-well plates at a concentration of 2×10^5 cells/well and incubated overnight with serum-free DMEM. On the next day, the seeded cells were treated with the IC_{50} value of each crude extract and incubated for 24 hrs. The cells were harvested and washed with PBS. The cells (1×10^5) were resuspended in 195 µL Binding solution and stained with 5 µL FITC Annexin V and 10 µL PI for 20 min at room temperature and then analyzed by a flow cytometry machine (BD FACS Aria II).

A fluorescent assay was performed using the TUNEL apoptosis kit (Beyotime, China). The cells treatment was similar to the flow cytometry tests except for seeding the cells in a 24-well plate. The cells were fixed with 4% paraformaldehyde for 20 min and washed with PBS 3 times. Fixed with PBS (with 0.3% Triton X-100) for 5 min once more. A 50 µL TUNEL working solution was added to each well and allowed to incubate for 60 min at 37°C. Then, the cells were washed with PBS 3 times and detected by microscope (Leica, DMI3000B).

***In vivo* anti-cancer tests:** GL261 cells were harvested and resuspended in PBS. The C57BL/6 mice were divided into 3 groups of ten mice each group randomly and each mouse was subcutaneously injected with 1×10^6 GL261 cells. Two weeks later, the tumour size and mice weight were measured by digital callipers and a weight scale and the tumour volumes were calculated by the formula:

$$V = \frac{ab^2}{2}$$

Where:
a = Tumor length
b = Tumor width

The XL-1325, MPT-426 and PBS (control) were injected into the tumour at different concentrations every 2 days when the tumour was the volume at 100 mm^3 (XL-1325: 2.5 mg kg^{-1}, MPT-426: 5 mg kg^{-1}) and tumour size was measured every other day. All the animals were euthanized after 2 weeks. The xenograft tumours were harvested and taken pictures. The animal experiments were proceeded by the Institution Animal Care (the animal ethical statement number: AMU WEC20201059).

Statistical analysis: Statistical analysis was performed using the SPSS 18.0 software (SPSS, IL, USA) and GraphPad Prism 6 (GraphPad, La Jolla, CA, USA). Animals were randomly assigned for drug treatment. Results are expressed as mean values±standard deviation (SD). The significant differences were assessed using the student's t-test and $p<0.05$ was considered significant (*$p<0.05$, **$p<0.01$ and ***$p<0.001$).

RESULTS

Crude extracts from the two endophytic fungi inhibit the proliferation of cancer cell lines: To validate the inhibitory effect of crude extracts *Chaetomium cochliodes* (XL-1325) on cancer cells, the IC_{50} values in Table 1 were obtained by using cancer cell line Hun-7 (0.62 μM), LN229 (0.67 μM), MDA231 (0.52 μM), MGC-803 (1.22 μM) and the crude extract *Penicillium* sp. (MPT-426) in Hun-7 (1.396 μM), LN229 (1.78 μM), MDA231 (3.424 μM), MGC-803 (1.1 μM). The ability to inhibit the proliferation of cancer cell lines was evaluated on Huh-7 and MGC-803 cell lines by CCK-8 assay. We treated Huh-7 and MGC-803 cells with the XL-1325 (2.5 μg mL^{-1}) and MPT-426 (5 μg mL^{-1}) or with the same dose of DMSO at different times (0, 1, 2, 3, 4 and 5 days). We observed that with crude extracts of *Chaetomium cochliodes* (XL-1325) and *Penicillium* sp. (MPT-426) treatment, the proliferation ability of cells decreased significantly on Huh-7 in Fig. 1a and b or MGC-803 cells in Fig. 1c and d compared with the control group. From day 1, the proliferation of Huh-7 and MGC-803 cells is stopped. As shown in Fig. 1a, the OD450 value (range: 0.2081-0.2551) of Huh-7 cells treated with XL-1325 on day 5 was much lower compared to the treatment with DMSO (range: 1.5244-2.0105). Similarly, the OD450 value (range: 0.2050-0.2152) of Huh-7 cells treated with MPT-426 was lower compared to the DMSO treatment (range: 0.8383-1.0370), in Fig. 1b. When the cells of MGC-803 were treated with the two crude extracts, a similar pattern was found after 5 days of culture. The OD450 value of cells treated with XL-1325 (range: 0.2021-0.2072), in Fig. 1c, MPT-426 (range: 0.2165-0.2222), in Fig. 1d was much lower compared to the control group (range: 1.0574-1.5441, 0.9685-1.1571) respectively. These results indicate that the crude extracts of *Chaetomium cochliodes* and *Penicillium* sp., can kill tumour cells effectively *in vitro*.

Crude extracts from the two endophytic fungi induce cell cycle arrests in cancer cell lines: The above studies confirmed that the crude extracts of *Chaetomium cochliodes* and *Penicillium* sp., can kill tumour cells *in vitro* and we further explored the influence on the cell cycle in both Huh-7 and MGC-803 cells. Compared with the control (DMSO) group in Fig. 2a, the cell cycle progression of MGC-803 significantly changed in Fig. 2b when treated with the crude extracts of *Chaetomium cochliodes* at the dose of 250 μg mL^{-1}. But the impact of the *Penicillium* sp., crude extract on the cell cycle is scarce in Fig. 2c. When treated with the crude extracts of *Chaetomium cochliodes*, the cell population of the S phase (average: 28.69%, range: 26.89-31.76%) is reduced concurrently with the increase of the G2/M phase population (average: 47.58%, range: 46.29-49.79%) compared with the control (S phase: Average is 53.43%, ranged in 52.29-54.87%, G2/M phase: Average is 17.38%, ranged in 13.78-20.53%), on average a 30% increase in G2/M phase and a 25% reduce in S phase in Fig. 2d. When treated with the crude extracts of *Penicillium* sp., the average population of cells is 7.79% (range: 7.26-8.23%) in the G2/M phase and 57.14% (range: 54.62-59.2%) in the S phase in Fig. 2d. The results suggested that the *Chaetomium cochliodes* crude extract mainly induced G2/M phase arrest of MGC-803 cells, which might be contributing to the apoptosis.

Table 1: *In vitro* growth inhibitory activity (IC_{50} μg mL^{-1}) of crude extracts

Crude extracts	Huh-7	LN229	MDA231	MGC-803
MPT-426	1.40±0.45	1.78±0.32	3.42±0.96	1.10±0.50
XL-325	0.62±0.05	0.67±0.29	0.52±0.14	1.22±0.82

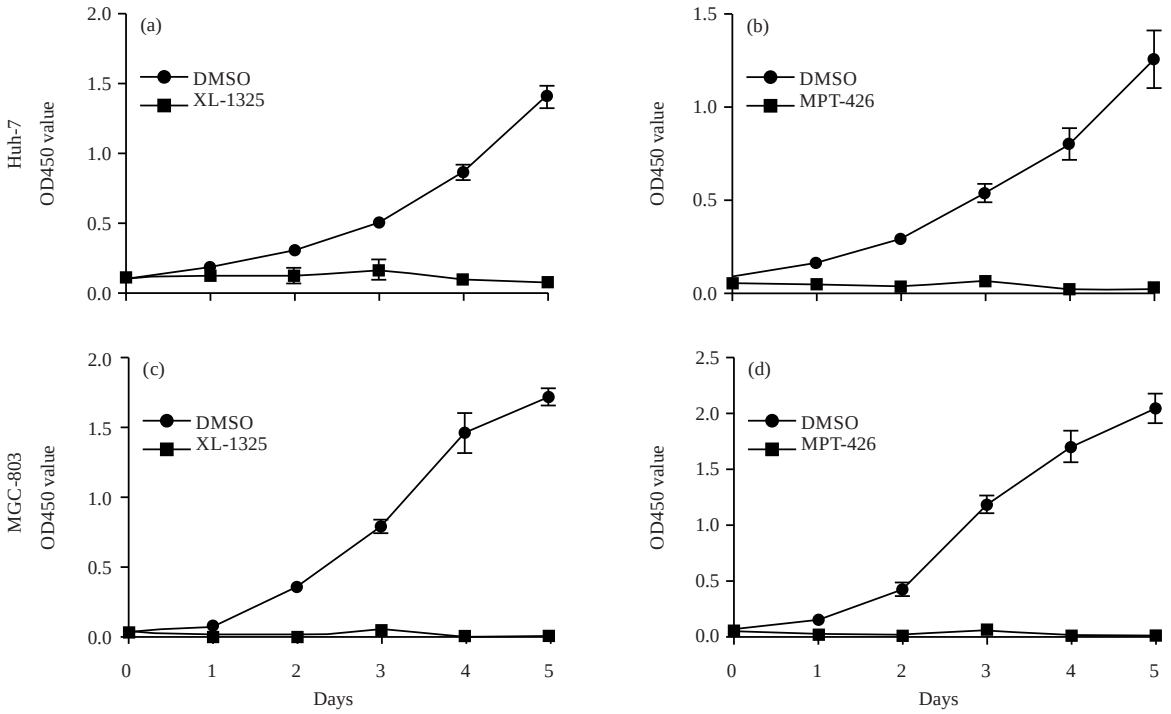

Fig. 1(a-d): Efficacy of the crude extracts from *Chaetomium cochliodes* (XL-1325) and *Penicillium* sp. (MPT-426) against the proliferation of human cancer cell lines, (a) Huh-7 cells treated with XL-1325, (b) Huh-7 cells treated with MPT-426, (c) MGC-803 cells treated with XL-1325 and (d) MGC-803 cells treated with MPT-426

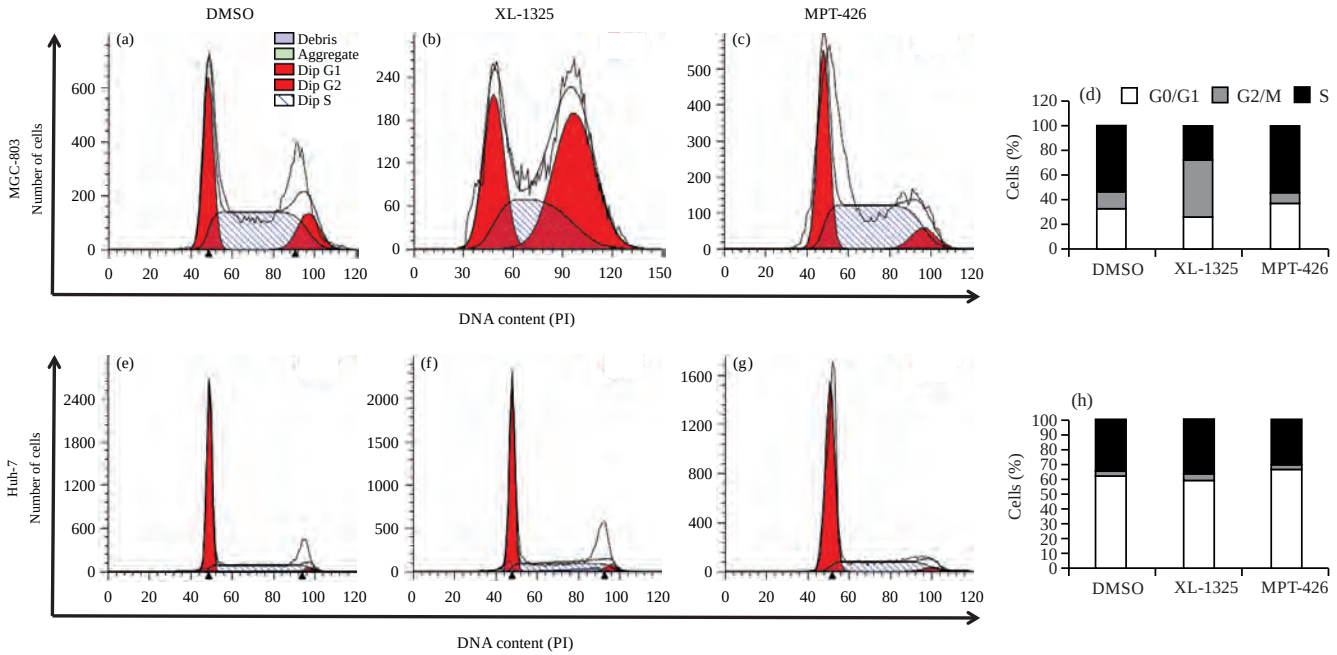

Fig. 2(a-h): Effect of crude extracts XL-1325 and MPT-426 on cell cycle of human cancer cell lines, (a) Cell cycle analysis of MGC-803 cells treated with DMSO (Control), (b) Cell cycle analysis of MGC-803 cells treated with XL-1325, (c) Cell cycle analysis of MGC-803 cells treated with MPT-426, (d) Percent cell cycle distribution of MGC-803 cells, (e) Cell cycle analysis of Huh-7 cells treated with DMSO (Control), (f) Cell cycle analysis of Huh-7 cells treated with XL-1325, (g) Cell cycle analysis of Huh-7 cells treated with MPT-426 and (h) Percent cell cycle distribution of Huh-7 cells

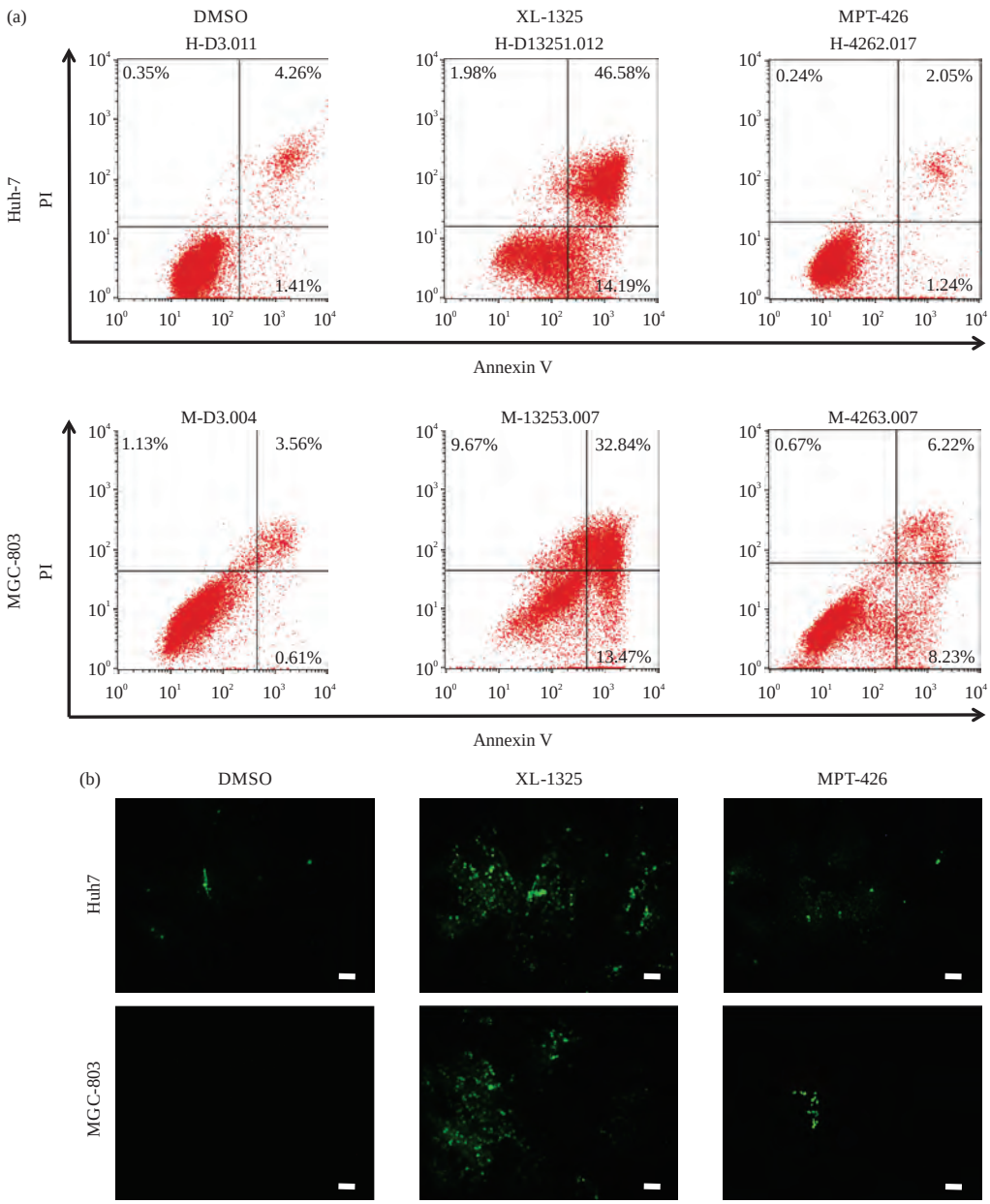

Fig. 3(a-b): Effect of crude extract XL-1325 and MPT-426 on apoptosis of human cancer cell lines, (a) Treated with the crude extracts of XL-1325 and MPT-426 for 24 hrs and (b) Fluorescent image of the effect on apoptosis in two human tumour cells (scale = 100 μm)

Ratio of early and late apoptosis of Huh-7 cells and MGC-803 cells can be assessed by Annexin V-FITC/PI double-staining. Green fluorescence indicates that cells of apoptosis are increased after the treatment with crude extracts

On the contrary, compared with the DMSO treatment in Fig. 2e, both the crude extracts of *Chaetomium cochliodes* in Fig. 2f and *Penicillium* sp., Fig. 2g showed slightly infection on the cell cycle change of the Huh-7 cells, on average a 63% in G0/G1 phase (range: 59.04-66.96%), a 3.44% G2/M phase (range: 1.85-6.74%) and a 34% in S phase (range: 29.71-35.9%) in Fig. 2h. Thus, the apoptosis of Huh-7 cells might be induced mainly by another mechanism.

Crude extracts from the two endophytic fungi induce apoptosis: To elucidate whether the crude extracts of *Chaetomium cochliodes* and *Penicillium* sp., decreases cell survival through the induction of apoptosis in tumour cells, the Annexin V/PI Flow cytometry assays were conducted in Huh-7 and MGC-803 cells. As shown in Fig. 3a, the percentage of apoptotic cells increased gradually with the Chaetomium *cochliodes* crude extract treatment compared with the

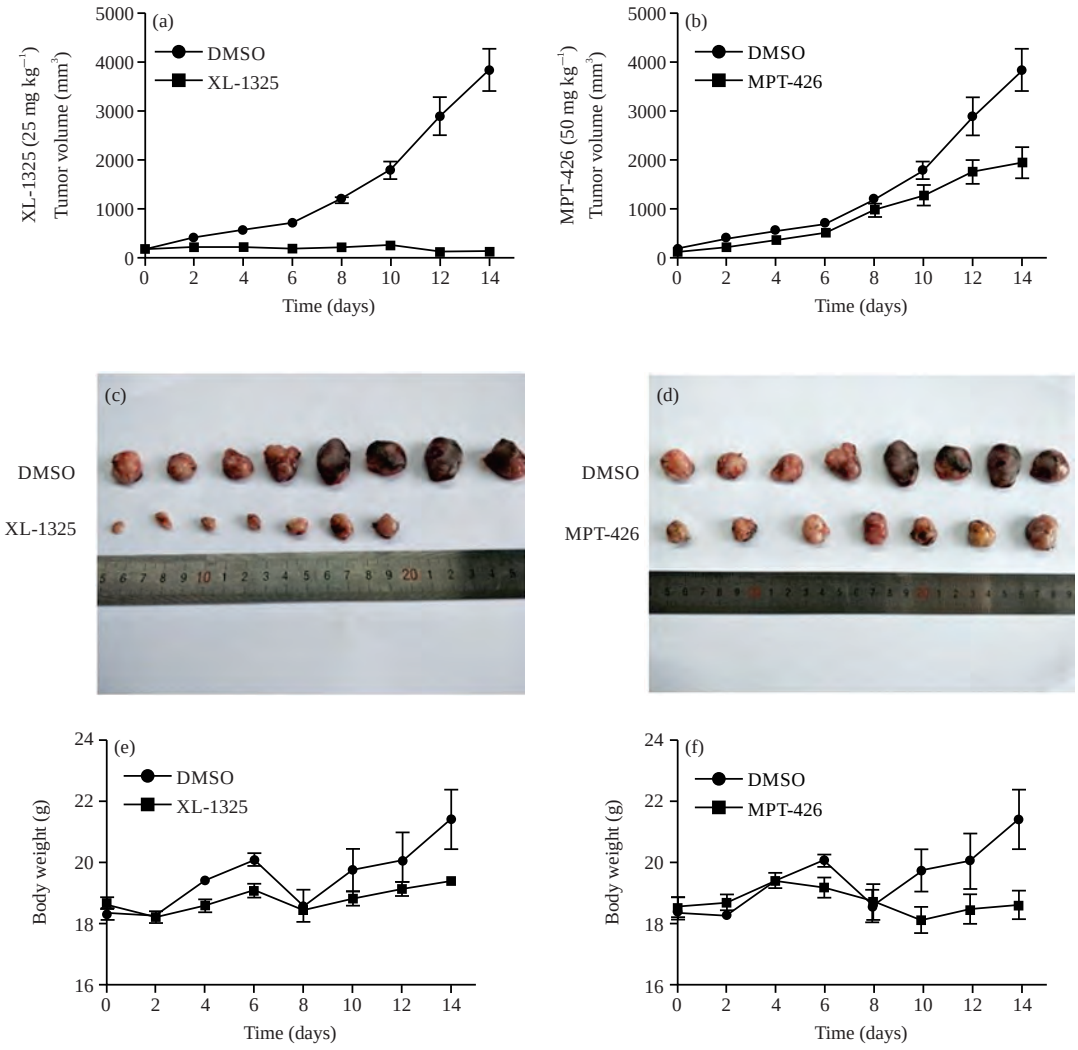

Fig. 4(a-f): *In vivo* anti-cancer tests of crude extracts XL-1325 and MPT-426, (a) Tumor growth curves for XL-1325 treatment and the control (DMSO), (b) Tumor growth curves for MPT-426 treatment and the control (DMSO), (c) Tumors excised and measurement as average±SD (mm^3), (d) Representative photographs of the tumours for MPT-426 treatment, (e) XL-1325 treatment and the control (DMSO) and (f) MPT-426 treatment and the control (DMSO)

Anti-cancer activity of the crude extracts was measured in C-57 mice, quantitative graphs of tumour inhibition rate were analyzed by the volume of the subcutaneous tumour and weight of the mice ($v = ab^2/2$), a: Tumour length, b: Tumour width and N = 8

control group. After 12 hrs of treatment with the *Chaetomium cochliodes* crude extract, the early apoptotic cell population increased gradually from 4.26±1.41% in the control group to 46.58±14.19% in Huh-7 cells and 3.56±14.19% to 32.84±13.47% in MGC-803 cells. The 12 hrs treatment with the *Penicillium* sp., crude extract, the early apoptotic cell population increased gradually from 4.26±1.41% in the control group to 46.58±14.19% in Huh-7 cells and 3.56±14.19 -32.84±13.47% in MGC-803 cells.

Then, the apoptosis degree was evaluated with TUNEL/PI staining kit. As shown in Fig. 3b, the fluorescence microscopy images show that the crude extracts of *Chaetomium cochliodes* and *Penicillium* sp., caused numerous positive staining of TUNEL, in comparison with the control. TUNEL/PI double-stained cells indicated that alterations have occurred in the cell morphology of apoptosis. The observation provided additional confirmation that the crude extracts of MPT-426 and XL-1325 induced the apoptosis of the cancer cells.

Crude extracts from the two endophytic fungi inhibit the growth of tumours in the mouse model: The above studies indicated that the crude extracts of *Chaetomium cochliodes* and *Penicillium* sp., inhibited cancer cell proliferation by

inducing apoptosis *in vitro*. To further investigate whether the extract could suppress tumour growth *in vivo*, GL261 (the murine glioma cancer cells) was transplanted into C57BL/6 mice. The crude extracts of *Chaetomium cochliodes* and *Penicillium* sp., significantly decreased the xenograft tumour size and volume compared with the control group (the weight barely change). The tumour size increased gradually from 173 mm^3 (range: 145-248 mm^3) to 3832 mm^3 (range: 2297-5162 mm^3) in the control group, decreased gradually from 180 mm^3 (range: 128-221 mm^3) to 130 mm^3 (range: 59-175 mm^3) in XL-1325 treatment in Fig. 4a and decreased gradually from 118 mm^3 (range: 73-192 mm^3) to 1956 mm^3 (range: 958-2467 mm^3) in MPT-426 treatment in Fig. 4b. Representative photographs were shown in Fig. 4c for XL-1325 treatment and in Fig. 4d for MPT-426 treatment, respectively. Comparatively, the weight of the mice in the XL-1325 treatment (19.4 g with the range from 18.3-20.6 g, in Fig. 4e and MPT-426 treatment (18.6 g with the range from 16.7-20.7 g) in, Fig. 4f slightly decreased compared with the control group (20.4 g with the range from 18.4-25.1 g). The results suggested that the two extracts might be active anti-cancer reagents.

DISCUSSION

Traditional Chinese medicine books "Zhong Cao Tui Xin" and "Chinese Medicine Dictionary" have recorded the functions of *Taxus* on diuresis, menstrual, hypoglycemic and kidney diseases. Recent studies also showed multiple medicinal properties of *Taxus* on anti-inflammatory and analgesic activities, anticonvulsant, antipyretic activities, antibacterial and antifungal activities[18]. After the discovery of taxol (Paclitaxel), the effective anticancer drug from the bark of Pacific yew in 1971, lots of work was carried out on the crude extracts[19,20] and compounds[21] investigation of several yew species. However, the slow growth of *Taxus* and the extremely low paclitaxel content limit its long-term development. Therefore, researchers must explore new production options, such as the search for paclitaxel or similar anticancer drugs from endophytic fungi.

It was the first reported that Paclitaxel was produced by fungal entophytes associated with Pacific Yew Tree in 1993[22]. Lots of strains of endophytic fungi were isolated from Yew trees later and found to produce Taxol[23-26] or anticancer crude extracts[27,28]. In the present study, we successfully isolated one endophytic fungus from Taxus and proved the crude extracts have obvious cytotoxicity in cancer cells and *in vivo*. It was further identified as belonging to the genera of *Penicillium*. Except for producing the famous antibiotic-penicillin, some members of the *Penicillium* genus have anticancer activities[29]. The ethyl acetate extract of *Penicillium cyaneum* has anticancer activity against HepG2 cells with the IC_{50} of 242.24 μg mL^{-1}. Moreover, Vasundhara *et al.*[28] isolated an endophytic fungus *Fusarium tricinctum* from *Taxus baccata* and tested their anticancer activities in MCF-7 and HeLa cell lines and resulting in IC_{50} values of 225 and 220 μg mL^{-1}, respectively. In this study, the IC_{50} values of the extracts from *Penicillium genera* in four cancer cell lines were 1.1-3.424 μg mL^{-1}, respectively. These results showed that our endophytic fungi strains have high cancer cells cytotoxicity.

Panax notoginseng is a traditional Chinese herb. The roots, leaves, fruit and flowers were all used as medicine against bleeding, chest pain, strokes etc. Endophytic fungi from *Panax notoginseng* were systemically studied and showed activity in antibacterial[30] and anti-inflammatory[31]. However, only limited evidence showed compounds or extracts from the fungal entophytes associated with *Panax notoginseng* have anticancer activity *in vitro* cancer cell lines[32], with the IC_{50} value of 3.5-13.4 μg mL^{-1}. In this study, the IC_{50} values of the crude extracts of strain XL-1325 (identified as *Chaetomium cochliodes*) varied from 0.52- 1.22 μg mL^{-1} in 4 cancer cell lines (Table 1), which showed extremely high anticancer activity both *in vitro* and *in vivo*.

Taxus media and *Panax notoginseng* are both widely used traditional Chinese herbs. With the development of new techniques, such as fungal culture and compound purification, more researches are focused on the fungal endophyte associated with those herbs. The two extracts from *Taxus media* and *Panax notoginseng* showed different toxicity on cancer cells and anti-cancer activity in mice in our study. The future study will be focused on the isolation of the active molecules from the extracts and their specificity on different cancer cells.

CONCLUSION

Results findings from *in vivo* mice model tests showed that both crude extracts of MPT-426 and XL-1325 inhibited the tumour growth but XL-1325 could inhibit the tumour growth more efficiently with a lower concentration than MPT-426. In summary, current results indicated that the crude extracts from the endophytic fungi *Chaetomium cochliodes* and *Penicillium* sp., could inhibit the cancer cell growth, induce the cell apoptosis and suppress the tumour growth *in vivo*.

SIGNIFICANCE STATEMENT

Overall, current research proved that the endophytic fungi strains of *Chaetomium cochliodes* from *Taxus media* and *Penicillium* sp., from *Panax notoginseng* would provide a basis for the identification of new, high-activity anticancer compounds.

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