Antimicrobial and Cytotoxic Potential of Crude Extracts from Culturable Fungal Endophytes Associated with the Mangrove Species Rhizophora stylosa and R. mucronata in the South China Sea

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Research Article

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Antimicrobial and Cytotoxic Potential of Crude Extracts from Culturable Fungal Endophytes Associated with the Mangrove Species *Rhizophora stylosa* and *R. mucronata* in the South China Sea

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Abstract

Our research evaluated the antimicrobial and cytotoxic activities of crude extracts of endophytic fungi from *R. stylosa* and *R. mucronate*. 46 fungal isolates were cultured on the four different mediums, namely Dextrose Agar (PDA), Czapek's Agar (CZA), Rice Medium (RM) and Grain Medium (GM), and harvested by ethyl acetate solvent at 40 days. The extracts were tested for antimicrobial activity by the microdilution method against Gram-negative bacteria *Pseudomons adaceae* (PA), Gram-positive bacteria *Enterococcus faecalis* (EF), *Methicillin-resistant Staphylococcus aureus* (MRSA) and pathogenic fungus *Monilia albican* (MA). Cytotoxic activity of the extracts was evaluated by MTT assay using human lung cancer cells A549, human cervical carcinoma cells Hela, and human hepatocellular cells HepG2. The results showed that rice medium could promote the secretion of antimicrobial and anti-tumor secondary metabolites of endophytic fungi in comparison with the other cultivation media. 17 strains (68%) from *R. stylosa* exhibited inhibition effects on indicators, especially *N. protearum* HHL46 which could inhibit the growth of four microbes with the MIC values reaching 0.0625 mg/mL. 15 strains (71.4%) from *R. mucronata* displayed activities against human pathogenic microbes, especially *Pestalotiopsis sp.* HQD6 and *N. protearum* HQD5 which could resist the growth of four microbial with MIC values ranging from 0.015 to 1 mg/mL. In cytotoxic assay, the extracts of 10 strains (40%), 9 strains (40%) and 13 strains (52%) from *R. stylosa* and 13 strains (61.9%), 10 stains (47.6%) and 10 stains (47.6%) in *R. mucronata* displayed the cytotoxicity against A549, Hela and HepG2 cancer cells with cells viability value ≤50%, respectively.

Neopestalotiopsis protearum HHL46, *Phomopsis longicolla* HHL50, *Botryosphaeria fusispora* HQD83, *Fusarium verticillioides* HQD48 and *Pestalotiopsis sp.* HQD6 displayed significant antitumor activity with IC_{50} values below the 20 μg/mL. These results highlighted the antimicrobial and anti-tumor potential of endophytic fungi from *R. stylosa* and *R. mucronata* and the possibility to be exploited for their antimicrobial and cytotoxic agents.

Keywords

R. stylosa; *R. mucronata*; Endophytic Fungus; Antimicrobial Activity; Cytotoxic Activity

Introduction

Mangrove forests are complex ecosystems that distributed in the intertidal zone of tropical and subtropical coasts, which not only refers to mangrove plants, but also include the diverse groups of microorganisms formed by abundant biological communities[1]. There are 61 species of true mangrove plants in the world, belonging to 14 families and 21 genus. China has 26 species, and 24 of them have been documented in Hainan[2]. Mangrove endophytic fungi tolerant to numerous stresses, such as high salinity, high temperature, extreme tides, oxygen pressure, high humidity, and light and air limitations, have evolved unique metabolic pathways for the purposes of the competition of nutrition and space in extremely fierce niche, which will inevitably render them capable of exhibit excellent biological activity *via* producing impressive numbers of metabolites with special biological functions[3, 4]. Rivai *et al* studied the antimicrobial activities of endophytic fungi from *R. mucronata* and ethyl acetate extract of 14 strains (64.3%) showed activity on test microbes of *S. aureus*, *E. coli*, and *C. albicans*[5]. *Cladosporium* sp. isolated from *Thespesia populneoides* and *Xylaria sp.* isolated from *Acanthus ilicifolius* were reported to exhibit Gram-positive and Gram-negative bacteria inhibition towards *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. Additionally, most extracts of 84 endophytic fungi from 10 different mangrove hosts belonging to seven families, which were Rhizophoraceae (*Rhizophora apiculata*, *R. mucronata*, *Ceriops decandra*), Sonneratiaceae (*Sonneratia alba*), Combretaceae (*Lumnitzera littorea*), Avicenniaceae (*Avicennia alba*), Acanthaceae (*Acanthus ilicifolius*), Meliaceae (*Xylocarpus granatum* and *Xylocarpus moluccensis*) and Malvaceae (*Thespesia populneoides*) and showed cytotoxicity against some cancer cell lines, including A375 (human malignant melanoma), SW620 (human colorectal adenocarcinoma), Kato III (human gastric carcinoma), HepG2 (human liver hepatoblastoma) and Jurkat (humanacute T cell leukemia)[6.] Extracts of 9 endophytic fungal isolates (64.3%) from *R. mucronata* can inhibit the growth of tested bacterium and fungi, of 12 isolates (85.7%) were cytotoxic (cell viability < 50%) against T47D cells[5].

Rhizophora is one of the most conspicuous genera of the most widespread mangrove family, the Rhizophoraceae. There were 66 genus endophytic fungi were isolated from plants of the genus *Rhizophora*, such as *Aspergillus*, *Cladosporium*, *Chaetomium*, *Fusarium*, *Lasiodiplodia*, *Penicillium*, *Pestalotiopsis*, *Phomopsis*, *Phoma*, *Phyllosticta*, *Trichoderma*, and more than 195 natural products, including alkaloids, terpenoids, coumarins, chromones, quinones, peptides, phenolic acids, lactones were identified, and cytotoxicity was found to be the most notable bioactivities of secondary metabolites isolated[2]. Among them, unprecedented scaffolds indole-diterpenes rhizovarins A-C isolated from *Mucor irregularis* QEN-189 in *R. stylosa*were against human cancer HL-60 and A-549 cell lines with IC_{50} values ranging from 5 to 15 μM and they are novel inhibitors of the Wnt/β-catenin pathway in breast cancer cells[7, 8, 9].A novel cytochalasin, which was found in endophytic fungus *Phomopsis sp.* derived from *Kandelia candel*, can effectively induce the apoptosis and inhibit the migration of human lung cancer cells A549 according to significantly increased the protein expression of Bax, p53 and cleaved Caspase-3, and increased ratio of the anti-apoptosis proteins of Bax/Bcl-2[10, 11.] In addition, demethylincisterol A_3 was as a selective inhibitor of a classical non-receptor protein tyrosine phosphatase Shp2 and isolated from *R. mucronata* endophytic *Pestalotiopsis sp.* HQD6[12, 13]. New polyketide derivatives pestalpolyol I was obtained from extracts of the endophytic fungus *P. clavispora* isolated from *R. harrisonii* exhibited strong cytotoxicity against the mouse lymphoma cell line L5178Y with an IC_{50} value of 4.10 μM[14].

During our previous work on biological potentials of endophytic fungi of mangrove-derived, we isolated and identified the endphytic fungi from the mangrove *R. stylosa and R. mucronata* and their antioxidant activities were evaluated[15]. In this study, we continuous investigated on antimicrobial and cytotoxic activities of endophytic fungi isolated from *R. stylosa and R. mucronat*. We tried to promote the secretion of antibacterial and antitumor substances of our fungal isolates by using four different culture medium. Four microbial indicator strains (PA, EF, MRSA and MA) and three human cancer cell lines (Hela, A549 and HepG2) were adopted for antimicrobial and cytotoxic tests, respectively. This study aims to provide complete information on the *in vitro* potential of the endophytic fungi of these two hosts as producers of antimicrobial and cytotoxic activities.

Results

Antimicrobial activity of fungal extracts

A total of 46 fungal extracts assayed, 32 extracts (69.6 %) showed antimicrobial activity against, at least, one of the indicator pathogenic microbes tested (Tables 1 and 2). The antimicrobial activity of the same isolated fungal strain was significantly different when cultured on kinds of mediums.

Of the endophytic fungi isolated from *R. stylosa* (25 isolates, Table 1), RM were determined to be more suitable for antibiotic production in fungal isolates than other three mediums. Of these, 13 strains cultured on RM (52%) exhibited antimicrobial activity using concentration of the extracts 10 mg/mL, and among them, 7 strains had stronger inhibitory effects on MRSA with MIC values less than 0.5 mg/mL. HHL55 showed the broadest antimicrobial spectrum against four indicator test microorganisms, CZA culture of HHL55 was found to show the most potent antimicrobial activity against PA with MIC value of 0.031 mg/mL. Only 2 strains fermented on the GM displayed low inhibitory activity with MIC value of 1 mg/mL. In addition, three culture media of HHL94, HHL64 and HHL82, showed inhibitory effect on three indicator test microorganisms.

Of the endophytic fungi isolated from *R. mucronata* (21 isolates, Table 2), most of the fungal strains (15 isolates, 71.4%) exhibited antimicrobial activity. A high growth inhibition rate were detected from the fungal extracts cultured on CZA (10 isolates, 47.6%) and RM (10 isolates, 47.6%), in comparison with PDA (7 isolates, 33.3%) and GM (2 isolates, 9.5%) at the concentration of 10 mg/mL selected. RM of 9 isolates and CZA of 3 isolates showed strong inhibitory effects on MRSA with MIC values less than 0.5 mg/mL. Only extract of HQD20 cultivated on the GM inhibited the growth the MRSA and MA. Moreover, HQD6 and HQD5 displayed antimicrobial activity against the growth of four indicator microorganisms tested with MIC values ranging from 0.015 to 1 mg/mL.

Cytotoxicity of fungal extracts

Cytotoxic effect of *R. stylosa* and *R. mucronata* endophytic fungal extracts were tested against HeLa, A549 and HepG2 cells using MTT colorimetric assay. Half inhibitory concentration (IC_{50}) values were shown in Table 3 and 4. Results are represented as the means ± standard deviation of experiments performed triplication. Cell viability using different concentrations of these extracts after 24 h of treatment were determined (Fig. 1 and 2). The cytotoxicity of fungal extracts showed a dose-dependent relationship.

As shown in Table 3, of the endophytic fungi isolated from *R. stylosa*, extracts of 10 isolates (40%) tested were cytotoxic and exhibited a percentage of viability A549 cells value ≤50%. Four extracts (HHL75, HHL46, HHL10 and HHL50) IC_{50} values were lower than 100 μg/mL (IC_{50} values of 64.38±3.40, 11.65±0.34, 97.21±1.36 and 16.31±0.36μg/mL, respectively), suggesting the cytotoxic potential of these four fungal isolates (Fig. 1 A, B, C and D). Extracts of 9 isolates (36%) showed cytotoxicity and displayed a percentage of viability Hela cells value ≤50%. Especially, HHL46, HHL82, HHL52 and HHL50 extracts showed the most significant cytotoxic effect, with IC_{50} values of 31.03±1.21, 14.38±1.84, 70.55±1.37 and 73.18±1.64, which were lower than 100 μg/mL (Fig. 1 E, F, G and H). Extracts of 13 isolates (52%) showed cytotoxicity against HepG2 cells, amongst 7 extracts (HHL61, HHL75, HHL46, HHL10, HHL52 and HHL50) could significantly suppress the proliferation of HepG2 cells with IC_{50} below 100 μg/mL (Fig. 1 I, J and K).

As shown in Table 4, of the endophytic fungi isolated from *R. mucronata*, extracts of 13 isolates (61.9%) displayed cytotoxic activity on A549 cells, 10 isolates (47.6%) on Hela cells, and 10 isolates (47.6%) on HepG2 cells. Of which, 9 extracts (HQD83, HQD33, HQD28, HQD48, HQD41, HQD5, HQD1, HQD6 and HQD8) exhibited significant cytotoxicity against A549 cells with IC_{50} less than 100 μg/mL (Fig. 2 A, B, C and D). 4 extracts (HQD83, HQD48, HQD5 and HQD20) showed strong cytotoxic effect against the Hela cell line with IC_{50} values below 100 μg/mL (Fig. 2 E, F, G and H). 7 extracts (HQD83, HQD28, HQD48, HQD41, HQD5, HQD6 and HQD57) that were potent (IC_{50} < 100 μg/mL) against HepG2 cells (Fig. 1 I J K and L).

In an attempt to promote the antitumor substances production, four different media were adopted for the fungal isolates cultivation to activate the biosynthetic silence gene expression. We found that extracts of 1 isolate (HQD28) cultured on PDA, 1 isolate (HQD48) cultured on CZA, 3 isolates (HHL82, HQD52 and HQD6) cultured on RM and 4 isolates (HHL46, HQD48, HQD5 and HQD8) cultured on GM exhibited significant anti-proliferative activity against at least one of the tested carcinoma cells with IC_{50} < 20 μg/mL. GM culture of *R. stylosa* endophytic HHL46, RM culture of HHL61 and HHL82 were most effective against A549, Hela and HepG2 with IC_{50} values of 14.38±1.84 μg/mL, 23.17±4.26 μg/mL and 14.38±1.84 μg/mL, respectively (Table 3). The extracts from CZA culture of *R. mucronata* endophytic HQD48, RM culture of HQD20 and HQD6 exhibited the highest cytotoxicity towards A549, Hela and HepG2 with IC_{50} values of 4.83±1.61μg/mL, 14.38±1.84 μg/mL and 9.58±0.01 μg/mL, respectively (Table 4). The genus *Pestalotiopsis* and *Phomopsis* were demonstrated to be a rich source of antitumor secondary metabolites. Notable, RM culture of HQD6 exhibited cytotoxic and antiproliferative effects against three tested cancer cell lines with IC_{50} values ranged from 9.58 μg/mL to 14.99 μg/mL.

Discussion

Infectious diseases, including bacterial infections, pose a serious threat to global health and drug resistance[16, 17]. Unfortunately, there has been resistance detected against every antibiotic on the market and if antibiotic resistance is not remedied pathogenic bacteria will once again become one of the leading causes of mortality with an estimated yearly death toll of ~10 million by 2050[18]. The development of new antimicrobial agents is pointed out as one of the effective solutions to address this problem[19, 20]. As a promising source of diverse and structurally unprecedented bioactive natural products, mangrove-derived endophytic fungi are unquestionable continuously attracted considerable attention[21, 22]. In our current study, of 46 endophytic fungi strains investigated, 32 extracts (69.6 %) exhibited antimicrobial activity, which is in consistent with the findings of previous study of Buatong *et al.*[23].that 61.3% mangrove fungal endophytes produced inhibitory compounds.

Cancer-related death is one of most the significant threats to human health worldwide, it is estimated 12.7 million new cases and 7.6 million cancer death each year[24]. At present, the primary treatment method for cancers is combine removing the tumour with the anesthetic agents after surgery[25]. Unfortunately, after the surgery process, it is possibly leads to tumour progression cause a large number of tumour cells to be released and due to reducing the activity of T, B and NK lymphocytes in the postoperative period[26]. It has also generated a large body of information that is being harnessed to develop new therapeutic modalities for treating cancer[27], however, search for cytotoxic agents selectively impact on proliferating cells still play an essential role in tumour treatment[28]. In our study, of 46 endophytic fungi strains investigated, 23 extracts (50%) showed cytotoxicity more or less, amongst 21 extracts (46.65%) were cytotoxic against A549 cells, 16 extracts (34.78%) were cytotoxic against Hela cells, 21 extracts (46.65 %) were cytotoxic against HepG cells. That were in accordance with the previous reports, of which 9 endophytic fungi were successfully obtained from the leaves of *Ginkgo biloba*, the extracts of isolates J-1, J-2 and J-3 markedly inhibited the proliferation of HeLa cells, promoted their apoptosis and blocked their migration[29] and 12 isolates extracts (85.7%) derived from mangrove *Rhizophora mucronata* were cytotoxic (cell viability < 50%) against T47D cells[5].

Culture-dependent methods have been developed aimed at substantial increases of biological active secondary metabolites production by any given microorganism [30]. RM was demonstrated to have the highest suitability for antibiotic production which 23 extracts (50%) were showed antimicrobial activity against at least one of four strains and the extract of HQD1 exhibited antimicrobial activity against MRSA with MIC value of 0.031 mg/mL, which were in accordance with the previous reports of Rivai *et al*[5]. RM (11 isolates, 39.13%) and GM (9 isolates, 19.56%) were more suitable for antitumor agent production, which agreed with previous reports of GM cultivated *Oidiodendron truncatum* led to the discovery of potent anticancer agent chetracins B with cytotoxicity against five human cancer lines reaching nM degree[31]. The variation in the antibiotic and cytotoxic properties among media could possibly be related to the composition of RM and GM activated our isolated fungal biosynthetic gene clusters[32].

Materials and methods

Fungal material used. Previously isolated endophytic fungi from healthy roots, stems, leaves, hypocotyls and flowers of *R. stylosa* and *R. mucronata* collected from a specific location (110°32′-110°37′ E, 19°51′-20°01′ N) in Dong Zhai Gang-Mangrove Garden on Hainan Island, China, were used for the present study. These endophytic fungi were identified in combination of morphologic characteristics and p internal transcribed spacer (ITS) sequences. Their antioxidant capacity have also been demonstrated[15].

Fermentation and extraction. Fungal isolates were cultured on Petri dishes of potato dextrose agar (PDA) at 28 °C for 5 days and then inoculated on four different mediums such as PDA, Czapek’s Agar (CZA), Rice Medium (RM), Grain Medium (GM) for stationary fermentation. After 40 days, the harvested cultures were extracted by 100 mL the ethyl acetate and treated in ultrasound at 50℃ for 1 h, then, filtrated by filter paper. Rotary evaporator was used at 50 ℃ with low pressure to evaporate the remaining of ethyl acetate. The extracts were dissolved in dimethyl sulfoxide (DMSO, not exceed 1%, v/v) and stored at 4℃ until being used.

Screening for antimicrobial activity. The endophytic fungal extracts were tested against Gram-negative bacteria *Pseudomons adaceae* (PA), Gram-positive bacteria *Enterococcus faecalis* (EF), *Methicillin-resistant Staphylococcus aureus* (MRSA) and pathogenic fungus *Monilia albican* (MA) by microdilution method with some modifications[33]. An aliquot of extract (2 μl) was added to 198 μl of the indicator strain suspension with a density of 5×10^6 CFU/ml into each well of a 96-well microplates. This mixture was then incubated for 24 h/48 h at 37°C/28°C and the minimum inhibitory concentration (MIC) were recorded. Amphotericin B was used as positive control.

Screening for cytotoxicity. Cytotoxicity was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method according to our previous report[13]. Human cervical carcinoma cells (HeLa), human lung cancer cells (A549), human hepatoma cells (HepG2) cells were grown in RPMI-1640 culture medium with 200 μL/mL fetal bovine serum (FBS) under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. 100μL cell suspension at a density of 1.5×10^5 cell mL^{-1} was pipetted into 96-well microtiter plates. Fungi extracts with different concentrations from 100 μg/mL to 600 μg/mL were added to each well and incubated for 24 h at the above conditions in a CO_2-incubator. 20μL MTT (5 mg/mL) was added to each well and plates were further incubated for 3 h. 200 μL DMSO was added to dissolve the formazan crystals. The absorbance was then measured at 570nm by a microplate reader. The cell inhibition rate (%IR) was calculated by the equation as follows: % IR=$[(A_{bla}-A_{sam})/A_{bla}]\times100$, where A_{bla} is the absorbance of the blank, A_{sam} is the absorbance of the test compounds. The IC_{50} value was calculated from the dose-response relationship. Doxirubicin was used as the positive control.

Conclusion

To date, there are few systematic studies on the antimicrobial and antitumor potential on mangrove endophytic fungi. Our study indicated that the antimicrobial and cytotoxic activities of mangrove endophytic fungi extracts grown on four media showed distinguishable differences of activities and revealed the RM could promote the secretion of bioactive secondary metabolites. *Neopestalotiopsis protearum* HQD5 and *Pestalotiopsis sp.* HQD6 showed the potent antimicrobial activity, *Neopestalotiopsis protearum* HHL46, *Phomopsis longicolla* HHL50, *Botryosphaeria fusispora* HQD83, *Fusarium verticillioides* HQD48 and *Pestalotiopsis sp.* HQD6 displayed significant antitumor activity. Considering that, these fungi could be further explored for the characterization of antimicrobial and cytotoxic secondary metabolites which could explain the significant biological activities of the abovementioned fungal strain.

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Table 1 Antimicrobial acticity of the endophytic fungi from *R. stylosa*

Number	Species	MIC mg/mL															
		PDA[1]				CZA				RM				GM			
		M.A.[2]	MRSA.	P.A.	E.F.	M.A.	MRSA.	P.A.	E.F.	M.A.	MRSA.	P.A.	E.F.	M.A.	MRSA.	P.A.	E.F.
HHL70	*Botryosphaeria dothidea*	1	-	1	-	-	-	-	-								
HHL104	*Cladosporium cladosporioides*	-			-	-	-	-	-								
HHL55	*Cytospora rhizophorae*	-	1		1	-	0.0625	0.031	-		1		1		1	1	
HHL59	*Diaporthe ceratozamiae*	-			-	-	-	-	-		1						
HHL53	*Diaporthe eucalyptorum*	-			-	-	1	-	-	1	1						
HHL61	*Diaporthe perseae*	-			-	-	-	-	-	1	1						
HHL7	*Diaporthe sp.*				-	-	1	-	-								
HHL48	*Fusarium solani*	1	-		-	-	-	-	-								
HHL96	*Guignardia mangiferae*				-												
HHL31	*Lasiodiplodia ps eudotheobromae*				-												
HHL94	*Lasiodiplodia theobromae*		1		0.125		0.125	0.125									
HHL129	*Neofusicoccum mangiferae*		1								0.25						
HHL75	*Neofusicoccum parvum*				-						0.25						
HHL46	*Neopestalotiopsis protearum*			1	-	1	0.0625		0.062	1	0.125	0.5					
HHL82	*Pestalotiopsis microspora*				-		1	0.125		0.125	0.5	1		1	1		
HHL51	*Pestalotiopsis palmarum*				-												
HHL79	*Pestalotiopsis photiniae*				-						1						
HHL10	*Pestalotiopsis sp.*				-												
HHL56	*Pestalotiopsis theae*				-												
HHL22	*Phomopsis asparagi*				-					1							
HHL52	*Phomopsis glabrae*				-												
HHL50	*Phomopsis longicolla*				-	1	1			1	0.25						
HHL20	*Phomopsis sp.*				-												
HHL38	*Seiridium ceratosporum*				-						0.5						
HHL81	*Valsa brevispora*									1	0.25						

[1] Strains were cultivated on four different medium, which were Dextrose Agar (PDA), Czapek's Agar (CZA), Rice Medium (RM) and Grain Medium (GM) from *R. stylosa*

[2] Antimicrobial activites were tested against Gram-negative (*Pseudomons adaceae* (PA)), Gram-positive (*Enterococcus faecalis* (EF), *Methicillin-resistant Staphylococcus aureus* (MRSA)) bacteria and fungi (*Monilia albican* (MA))

Table 2 Antimicrobial acticity of the endophytic fungi from *R. mucronata*

Number	Species	MIC mg/mL															
		PDA[3]				CZA				RM				GM			
		M.A.[4]	MRSA.	P.A.	E.F.	M.A.	MRSA.	P.A.	E.F.	M.A.	MRSA.	P.A.	E.F.	M.A.	MRSA.	P.A.	E.F.
HQD24	*Aspergillus fumigatus*																
HQD83	*Botryosphaeria fusispora*																
HQD25	*Colletotrichum gloeosporioides*		1														
HQD62	*Diaporthe eucalyptorum*					1	-	-	1	1							
HQD33	*Diaporthe pascoei*	1															
HQD17	*Diaporthe phaseolorum*						0.062	1	1								
HQD29	*Diaporthe sp.*					1			0.25		0.125						
HQD28	*Eutypella scoparia*																
HQD48	*Fusarium verticillioides*				0.062			0.5									
HQD72	*Lasiodiplodia theobromae*		1		0.125		0.125	0.125									
HQD23	*Neofusicoccum mangiferae*		1								0.25						
HQD41	*Neofusicoccum parvum*										0.25						
HQD5	*Neopestalotiopsis protearum*			1	-	1	0.062		0.062	1	0.125	0.5					
HQD55	*Paraconiothyrium hawaiiense*																
HQD20	*Pestalotiopsis microspora*				-		1	0.125		0.125	0.5	1		1	1		
HQD1	*Pestalotiopsis protearum*					1					0.031						
HQD6	*Pestalotiopsis sp.*	1	1		0.015	1		1		1	0.031	1	1				
HQD57	*Phomopsis glabrae*																
HQD8	*Phomopsis longicolla*				-	1	1			1	0.25						
HQD47	*Pseudofusicoccum stromaticum*																
HQD22	*Valsa brevispora*									1	0.25						

[3] Strains were cultivated on four different medium, which were Dextrose Agar (PDA), Czapek's Agar (CZA), Rice Medium (RM) and Grain Medium (GM) from *R. mucronata*

[4] Antimicrobial activites were tested against Gram-negative (*Pseudomons adaceae* (PA)), Gram-positive (*Enterococcus faecalis* (EF), *Methicillin-resistant Staphylococcus aureus* (MRSA)) bacteria and fungi (*Monilia albican* (MA))

Table 3Anti-tumor activity of endophytic fungi from *R. stylosa*

NO.	Species	IC_{50}μg/mL											
		A549				Hela				HepG2			
		PDA	CZA	RM	GM	PDA	CZA	RM	GM	PDA	CZA	RM	GM
HHL70	*Botryosphaeria dothidea*												
HHL104	*Cladosporium cladosporioides*												
HHL55	*Cytospora rhizophorae*												
HHL59	*Diaporthe ceratozamiae*	-	-	140.46±5.61	-						26.54±3.73		
HHL53	*Diaporthe eucalyptorum*	-	-	-	-							185.03±3.22	
HHL61	*Diaporthe perseae*	-	-	201.04±1.22	-							23.17±4.26	
HHL7	*Diaporthe sp.*												
HHL48	*Fusarium solani*												
HHL96	*Guignardia mangiferae*												
HHL31	*Lasiodiplodia pseudotheobromae*												
HHL94	*Lasiodiplodia theobromae*												
HHL129	*Neofusicoccum mangiferae*												
HHL75	*Neofusicoccum parvum*	-	64.38±3.40	-	-		109.77±1.21				84.23±1.61		
HHL46	*Neopestalotiopsis protearum*	-	144.39±1.58	-	11.65±0.34			31.03±1.21				69.15±1.63	
HHL82	*Pestalotiopsis microspora*	-	-	123.35±1.29	-			14.38±1.84				237.47±1.75	
HHL51	*Pestalotiopsis palmarum*							142.77±1.97					
HHL79	*Pestalotiopsis photiniae*											224.42±1.43	
HHL10	*Pestalotiopsis sp.*	-	-	97.21±1.36	-							39.09±1.38	
HHL56	*Pestalotiopsis theae*												
HHL22	*Phomopsis asparagi*	-	-	170.84±1.99	-	341.14±1.37						137.13±1.71	
HHL52	*Phomopsis glabrae*							70.55±1.37			33.04±1.21		

HHL50	*Phomopsis longicolla*	192.96±2.62	108.53±2.09	-	16.31±0.36	137.73±2.53	120.38±1.79	73.18±1.64			55.05±1.92	177.71±4.76	
HHL20	*Phomopsis sp.*	-	-	605.5±1.51	-			190.74±1.39				378.08±1.59	
HHL38	*Seiridium ceratosporum*	386.35±3.82	151.1±2.25	-	-					239.82±1.89			
HHL81	*Valsa brevispora*								277.67±2.02				

Table 4 Anti-tumor activity of endophytic fungi from *R. mucronata*

NO.	Species	IC50μg/mL											
		A549				Hela				HepG2			
		PDA	CZA	RM	GM	PDA	CZA	RM	GM	PDA	CZA	RM	GM
HQD24	*Aspergillus fumigatus*		179.67±1.21										
HQD83	*Botryosphaeria fusispora*	248.17±1.22	62.63±1.63	208.13±1.42	112.17±1.63		30.62±1.21			223.05±1.51	67.73±1.22		262.05±1.45
HQD25	*Colletotrichum gloeosporioides*	144.89±1.71								241.22±6.19			
HQD62	*Diaporthe eucalyptorum*											182.03±1.32	
HQD33	*Diaporthe pascoei*	109.64±1.25	23.62±1.96				214.44±1.57						
HQD17	*Diaporthe phaseolorum*					201.66±1.12							
HQD29	*Diaporthe sp.*				331.91±1.33	498.96±1.66							
HQD28	*Eutypella scoparia*	53.81±1.37			277.58±1.30					14.75±1.21		227.03±1.26	
HQD48	*Fusarium verticillioides*	547.42±1.65	4.83±1.61	34.57±1.25	106.79±1.33		66.78±1.72		19.83±1.13	237.56±1.2	60.8±1.29	201.75±1.47	
HQD72	*Lasiodiplodia theobromae*												
HQD23	*Neofusicoccum mangiferae*												
HQD41	*Neofusicoccum parvum*		64.38±1.23				109.77±1.22				84.23±1.63		
HQD5	*Neopestalotiopsis protearum*		144.40±1.75		11.65±1.03			31.03±1.21				69.15±1.63	
HQD55	*Paraconiothyrium hawaiiense*												
HQD20	*Pestalotiopsis microspora*			126.73±1.21				14.38±1.84				237.47±1.75	
HQD1	*Pestalotiopsis protearum*		40.09±1.39		119.65±1.38		186.85±1.67						
HQD6	*Pestalotiopsis sp.*	28.90±1.26		14.99±1.62						196.17±1.65		9.58±0.01	
HQD57	*Phomopsis glabrae*										33.04±1.21		

HQD8	*Phomopsis longicolla*	192.97±1.86	108.53±2.21		16.31±1.26				
HQD47	*Pseudofusicoccum stromaticum*								
HQD22	*Valsa brevispora*								277.67±1.21

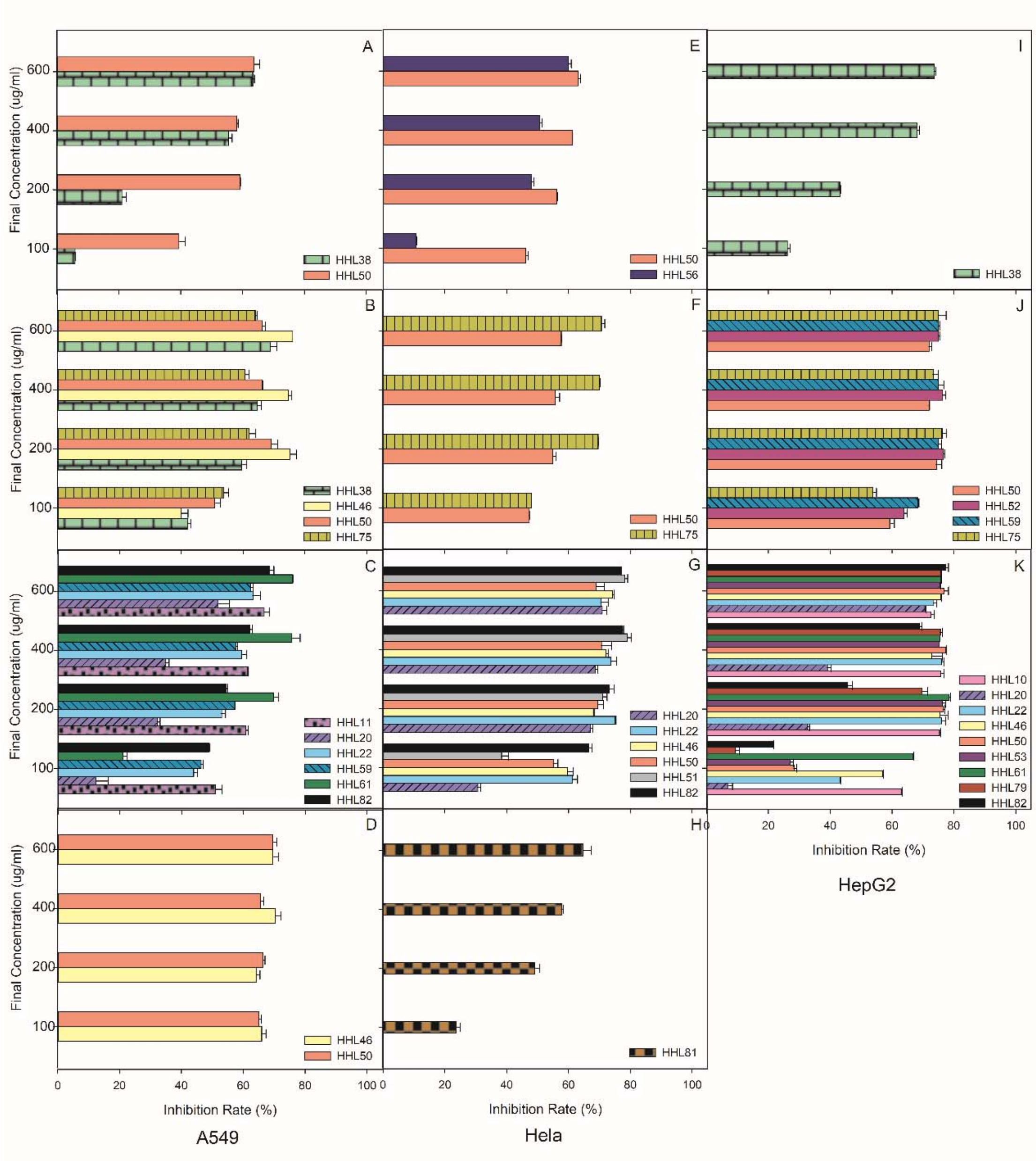

Fig. 1Anti-tumor activity of endophytic fungi from *R. stylosa*

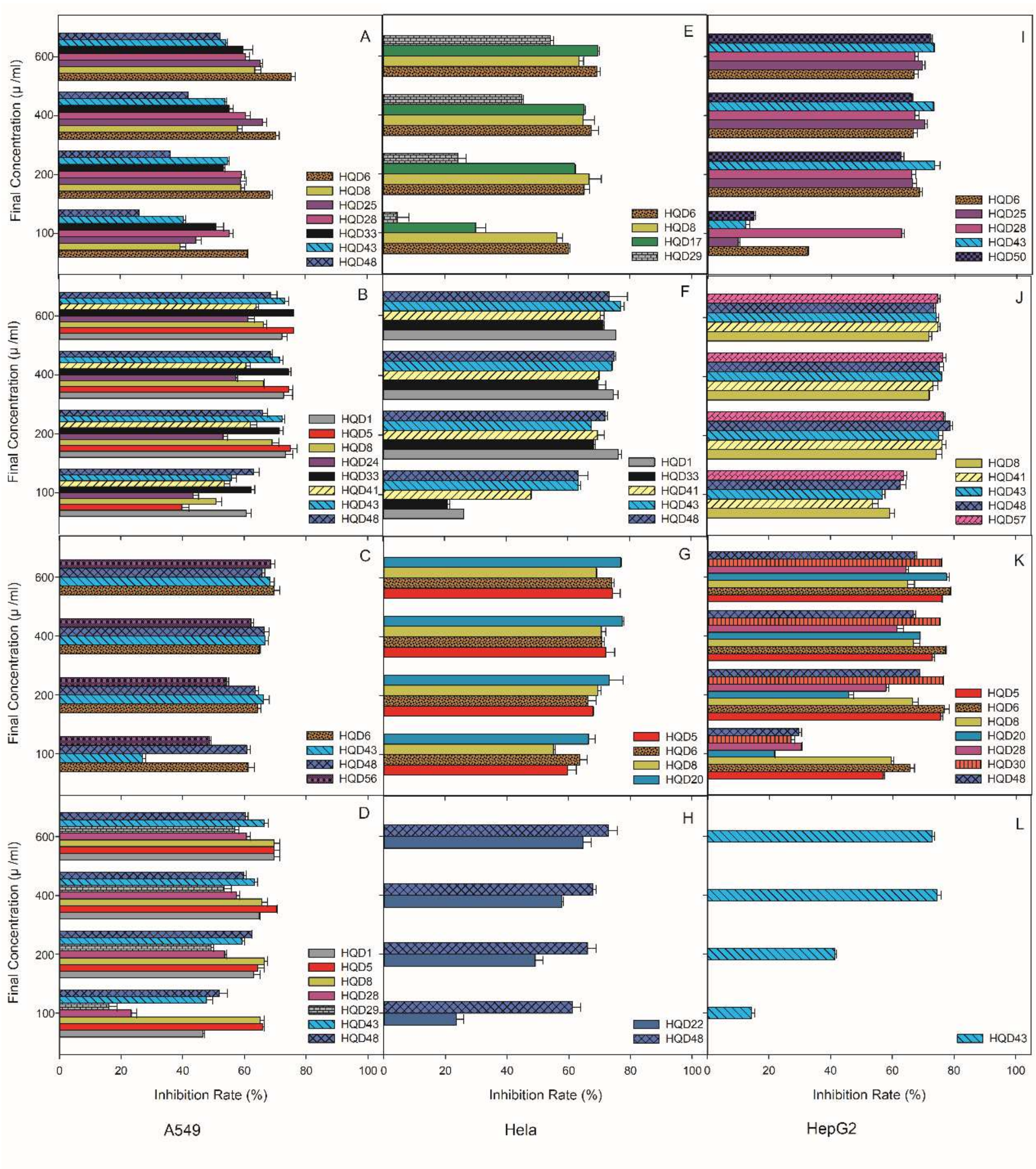

Fig. 2 Anti-tumor activity of endophytic fungi from *R. mucronata*

Figures

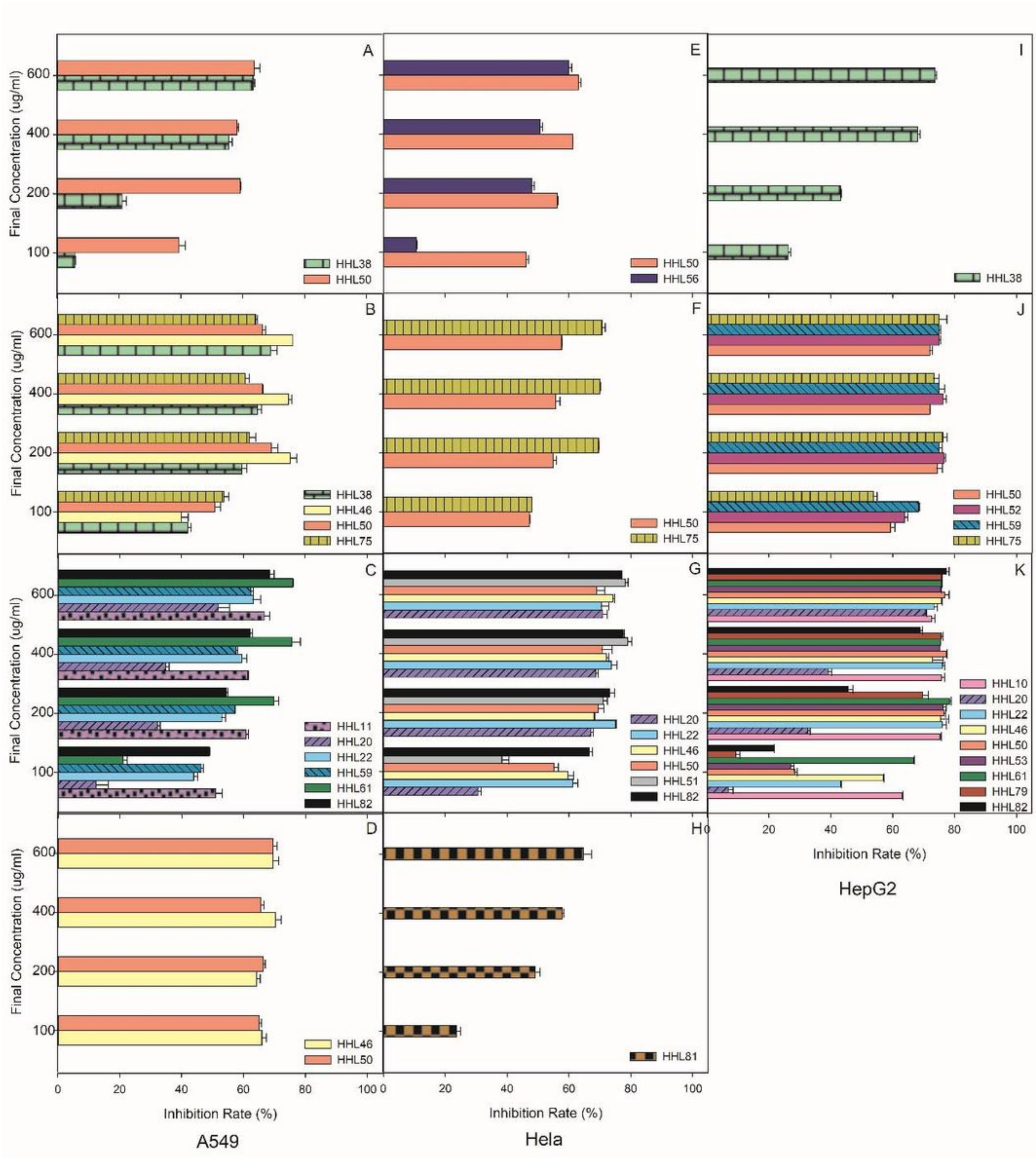

Figure 1

Anti-tumor activity of endophytic fungi from R. stylosa

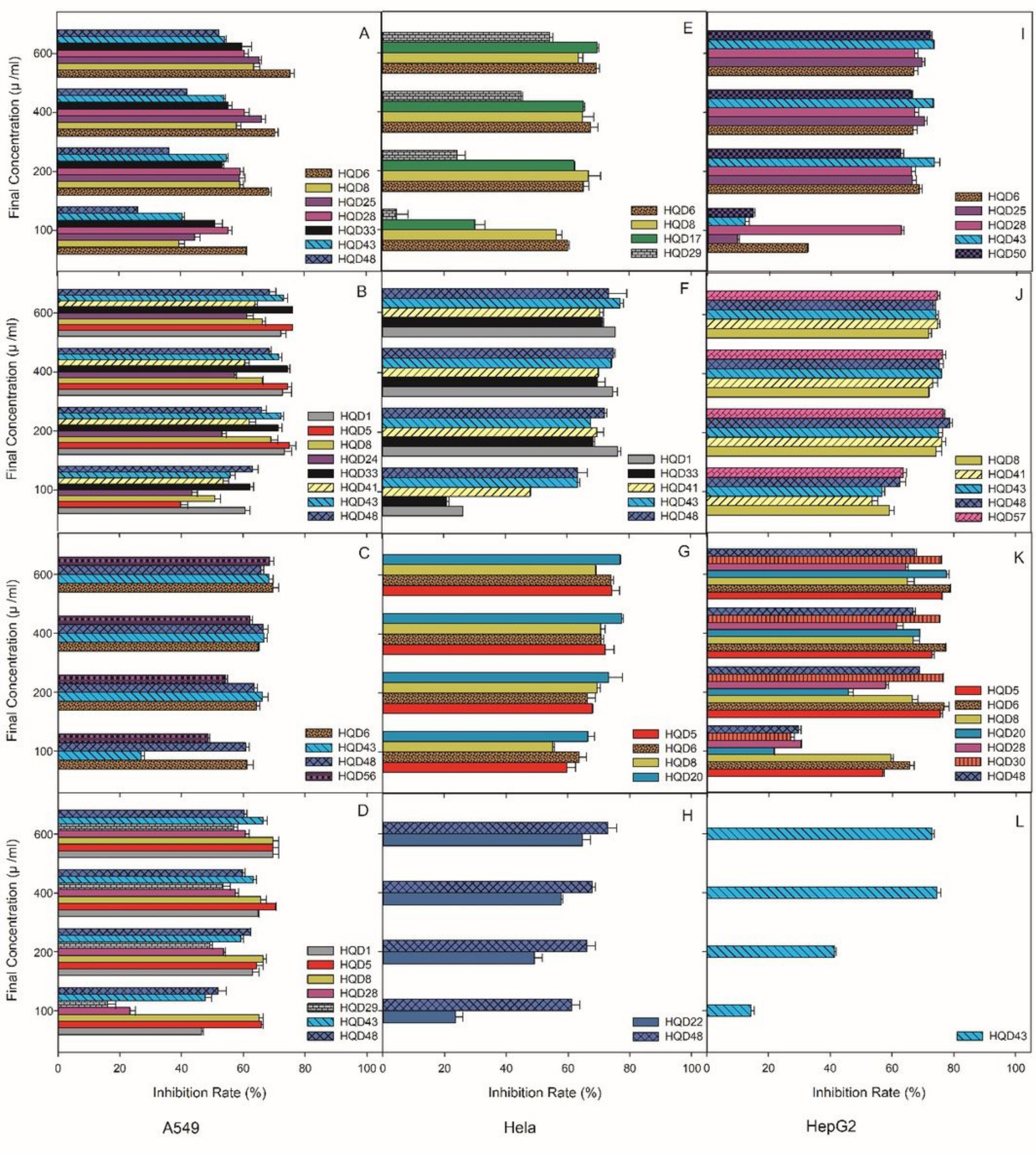

Figure 2

Anti-tumor activity of endophytic fungi from R. mucronata